

Two cheers for small space missions

Europe's achievements in space combine excellent science with responsible management. New measures to increase cost-effectiveness can only be welcome, while the United States can recover its shaky reputation by taking the lead in small missions.

OVER the past 20 years, two of the most expensive centres of European scientific excellence have been models of good management. Despite political buffeting, the European Laboratory for Particle Physics (CERN) and the European Space Agency (ESA) have consistently achieved ambitious scientific and technological goals while making hard decisions in controlling costs. Unfortunately, past successes are irrelevant when member states are determined to reduce the costs of participation, whereas past failures hang around the necks of such organizations for years.

It is important to distinguish between two types of external criticism of ESA and CERN. One type comes from those who are outsiders both institutionally and in spirit. The UK government is well established in this category — witness its unjustified claim that ESA's costs could be reduced by 25 per cent without damaging the science. The brute force of such an assertion can be valuable in concentrating minds, even if it is mistaken. Just as effective can be the critical scrutiny of outsiders who are insiders in spirit. One such is Professor Michael Cruise, of the University of Birmingham, England, who, responding to a need to reduce the estimated costs of a proposed mission in which he is involved to an acceptable level, has scrutinized the way in which ESA's mission costs arise. Cruise's analysis achieved a notable impact at a meeting last week of scientists involved in five such 'medium' mission proposals (costing up to US\$350 million or so), one of which will be selected in April. It was made clear that ESA will use the next medium mission as a pilot project in the application of his proposed cost reductions.

Space scientists are generally respectful of ESA's managers' capacity to work effectively within their budgets. Nevertheless, it is clear from Cruise's study that a trimming of ESA's practices is overdue — examples are the over-generous degree of provision of equipment such as computers, contracts with industry that place too little of the burden of unanticipated costs on contractors and opportunities to tighten the specification of missions early in their planning. The savings of 10 per cent or more that can be expected from such measures will also reduce the costs of subsequent ESA science missions. But the fun will begin when similar scrutiny is applied to other programmes in ESA, where cost disciplines are perceived to be more lax.

The political (budgetary) attractions of medium missions and small missions (\$50 million or less) can be scientifically dangerous. As ESA's latest big (or "cornerstone") mission, the Infrared Space Observatory (ISO) began to demonstrate last week when its first images emerged (see page 667), a major space-based observatory can provide large dividends for a variety of branches of astronomy. Those who pursue smaller missions at the expense of big ones — as seems to be happening in the United States — are misguided for that reason alone. Even more worrying is the suggestion by some US scientists that some of the small missions in NASA's Discovery programme (the first of which was launched last week) are driven more by a need to economize than to do good science.

ESA is not about to sacrifice big missions, and its cost reforms can only strengthen its external support. But the organization is about to reveal its limitations by abandoning any role with the smallest projects. Europe has a strong record of collaborations outside ESA, such as the AMPTE space-plasma project and the Rosat X-ray satellite. ESA had recently set aside funds to develop a role

for itself in this arena. Because of budget cutbacks following ESA's ministerial meeting last year, those funds are likely to disappear. But that is also symptomatic of one of ESA's shortcomings — its political structure and its relationships with industry are too cumbersome to make small, cheap and prompt missions a feasible part of its programme. With last week's launch of the NEAR asteroid rendezvous mission, NASA has an opportunity to teach its European equivalent the virtues of smallness — provided the economic virtues of its Discovery programme are matched by the science. □

South Africa's progress

After long delays, government initiatives give tangible cause for hope in South Africa.

THE significant subsidy increases awarded to South Africa's universities this year are the first sign that its government of national unity has taken seriously the crisis prevailing in the tertiary education system (see page 666). The report of the National Commission for Higher Education on the future funding system for universities and technikons is eagerly awaited. In the short term, the introduction of a system to provide funding, or at least adequate security against loans, for indigent students remains a pressing priority. But in the longer term, the government needs to address the issue of how many places in each discipline it intends to subsidize: the existing system has resulted in unfettered growth in student numbers in arts and social sciences in the past 12 years, while numbers in science, medicine and engineering have increased much more modestly.

The research community has understandably become frustrated about the lack of progress since the new government took over in May 1994. But here too, at last, there are signs of a new lease of life. The green paper (discussion document) released last month by Ben Ngubane, the Minister of Arts, Culture, Science and Technology, has the modest virtue of being not too prescriptive, while placing research firmly within the context of the reconstruction and development programme. With the appointment of Khotso Mokhele as president of the Foundation for Research Development — the government agency that funds research on both science and engineering in universities, technikons and museums — the community has an advocate with access to government who has good science at heart and the energy to pursue this ideal.

When President Nelson Mandela addresses the launch of the new National Academy of Science in Pretoria next month, he will probably try to persuade researchers that they should remain in South Africa to contribute their skills to reconstruction. It is important that they do so, but not only for reasons of secular patriotism. South Africa is the only country in Africa with both the infrastructure and the skilled manpower to stimulate the development that the continent so badly needs. The challenge to Mandela's government is to give effect to mechanisms that will encourage a renaissance of research in South Africa while at the same time ensuring that the science and engineering community becomes more broadly representative of society. □



Health ministry accepts liability in Japanese HIV-infected blood row

Tokyo. After more than six years of court battles, Japan's Ministry of Health and Welfare (MHW) last week finally accepted partial responsibility for failing to prevent the infection of thousands of Japanese haemophiliacs in the 1980s with HIV by contaminated blood products.

Last Friday (16 February), Naoto Kan, Japan's Minister of Health and Welfare, who has been pushing to resolve the blood product scandal since his appointment as minister last month, offered his "heartfelt apologies" directly to a large group of HIV-positive haemophiliacs and relatives of AIDS victims at a press conference at the ministry.

He added that, on behalf of the government, he "completely accepted" the views of Tokyo and Osaka district courts that "the government's delay in action magnified the damage". This apology and admission of

responsibility had been sought through court action by the haemophiliacs since 1989, but finally came after hundreds of HIV-positive haemophiliacs and their supporters staged a three-day sit-in in tents outside the MHW in Tokyo last week.

Kan's address marks the first time that the government has clearly accepted any responsibility for the disaster, in which two-fifths of Japan's 5,000 haemophiliacs were infected with HIV. The MHW took two years, from 1983 to 1985, to approve blood products heat-treated to kill HIV and other viruses, and even after approval of these products in July 1985 it failed to ban products that had not been heat-treated. During this period, large numbers of haemophiliacs were infected with HIV (see diagram over).

The plaintiffs decided to stage a sit-in outside the ministry after five companies that distributed the contaminated blood



Blood pressure: demonstrators mount a sit-in to force the government to admit mistakes.

coagulants — as well as the government, which had approved their use — refused to admit responsibility for allowing the disaster to spread. This was even after the Tokyo and Osaka courts urged them last October to pay large amounts of compensation to the victims in an out-of-court settlement.

The HIV-positive haemophiliacs — concealed within open tents in order to protect their privacy — were joined by large numbers of mostly youthful supporters, who held banners urging the ministry to admit its fault and apologize to the victims. Addresses to the crowd by victims and their families expressed sorrow at their loss, and anger at the government's continued recalcitrance.

Both the demonstration and Kan's apology follow the recent 'discovery' of files in the ministry building showing that health officials were aware that blood products were a possible route of HIV transmission in July 1983 (see *Nature* 379, 572; 1996).

Tetsu Noma, who is acting for the plaintiffs in the Tokyo court case, says that the discovery of the files, taken with Kan's apology, will have a "large effect" on the negotiations between the plaintiffs and the government, as they clearly establish the government's liability.

The biggest remaining barrier to a settlement, says Noma, is the unwillingness of two non-Japanese companies implicated in the scandal, Baxter Ltd and Bayer Yakuhin Ltd, to pay their share of the settlement proposed by the Tokyo and Osaka courts last October. The proposal suggested that the parties reach an out-of-court settlement in which each victim would be paid ¥45 million (US\$420,000), with 60 per cent of the cost to be met by the companies and the remainder by the government (see *Nature* 377, 467; 1995).

Negotiations over the settlement have progressed only slowly, as a political resolution is needed to meet the demands, says Noma. He claims that the government's budget is a key factor in any decision, as ►

Canadian inquiry points the finger

Montreal. More than 300 potential allegations of misconduct, contained in documents filed in federal court as a result of a commission of inquiry into Canada's contaminated blood scandal and made public last week, appear to form a searing indictment of the country's blood distribution system.

The allegations, which represent the provisional conclusions of the inquiry, headed by Mr Justice Horace Krever, point not just to possible neglect of duty among official bodies but sometimes to apparently deliberate malfeasance.

The Bureau of Biologics, for example, the body responsible for regulating and monitoring blood safety, is said by Krever to have failed to inform physicians and consumers of the risks of potentially contaminated blood products until 1988 — several years after such risks had been established. Indeed, it is also charged with approving potentially hazardous products as late as 1994.

According to Krever, another federal agency, the Laboratory Centre for Disease Control, may have distributed inaccurate and misleading information about AIDS while trying to reassure the public, according to court documents. The National Advisory Committee on AIDS, consisting of federally appointed scientists, purportedly allowed the public to be told in a pamphlet that the risk of

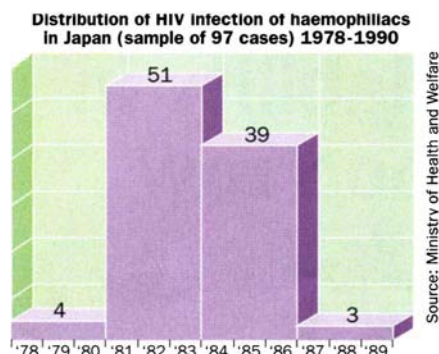
contracting HIV from a blood transfusion was only about two in a million — whereas in late 1985 it was already known to be as high as one in 166 for major surgery.

Some of Krever's most serious allegations are aimed at the Canadian Blood Committee. The committee is made up of senior federal and provincial health officials responsible for funding and overseeing the blood system, and Krever claims in his provisional conclusions that the committee was incapable of fulfilling its objectives, as its members had no pertinent expertise, no autonomy and insufficient resources.

The panel allegedly based the allocation of both money and plasma partly on political considerations, such as regional economic development needs, and devoted its resources to inadequate fractionation techniques, on which almost C\$700 million (US\$510 million) was wasted. The committee is also alleged to have hidden shortcomings in the regulation of blood products.

All the groups against which the allegations have been made have been given a chance to respond before Krever delivers his final verdict — though this is now expected to be delayed in the light of legal challenges to the procedure that Krever has adopted (see *Nature* 379, 479; 1996).

David Spurgeon



► the government's share of the original proposal made by the courts would be ¥32.4 billion (about US\$300 million) if compensation were offered to all 1,800 victims.

Apart from Kan's apology, there have been other indications that a resolution may be in sight. These include a recent announcement by Ryuichi Hashimoto, the prime minister, that a new compromise proposal to be issued by the Tokyo and Osaka courts at the end of this month will include measures to ensure the welfare of victim's families — a demand not dealt with in the previous proposal.

The government's admission of responsibility may also spur investigation into accusations of criminal liability and perjury that have been filed against some former ministry officials by the plaintiffs. Last week, the mother of a victim who died in 1991 filed an accusation of murder with the Tokyo District Prosecutor's Office against Takeshi Abe, who headed an MHW AIDS study group that advised continued use of non-heat-treated blood products in 1983.

The mother claims that her son was infected after receiving transfusions of coagulant from Abe in April and June 1985, well after Abe had learned in late 1984 that 23 out of 48 haemophiliacs receiving the same kind of blood products under his care had been infected with HIV.

The new charges follow an earlier accusation of "wilful negligence resulting in death" filed against Abe in 1994 (see *Nature* 368, 680; 1994), and a perjury charge filed last month against Atsushi Gunji, the head of the division within the ministry's pharmaceutical affairs bureau that set up the study group. Noma says that the newly discovered documents may prompt the prosecutors into action over these outstanding accusations.

Gunji, now a professor of public health at Tokyo University's Faculty of Medicine, has been accused of lying to the Tokyo district court in 1993 when he twice testified that ten years earlier he had been unaware that the transmission routes for HIV and the hepatitis-B virus were probably similar (see *Nature* 369, 388; 1996). The documents recently unearthed at the ministry, some written by Gunji, are said to contradict this testimony. But neither Gunji or Abe will comment publicly on the court cases, on the matter of the files, or on the accusations against them.

Stephen Barker & David Swinbanks

Scientists urged to turn their attentions to nuclear waste

Washington. The United States government is turning to basic science in an attempt to find new approaches to the massive task of cleaning up its nuclear weapons complexes. The Department of Energy (DoE) is inviting grant applications from scientists at universities and national laboratories for basic research into ways of simplifying the problem; the cost of the clean-up could be as high as \$1,000 billion. and it could take as long as 75 years.

Under the new programme, \$50 million will be made available this year in grants of between \$100,000 and \$300,000. The total will include \$20 million for university research groups, and \$20 million to support work in DoE laboratories. Critics claim that the funding level is far too low, given the scale of the problem; but the budget may grow in subsequent years.

"This is the first pure science programme we have undertaken," says Tom Grumbly, assistant secretary in charge of the DoE's Office of Environmental Management (OEM). Over the next few years, Grumbly predicts, the programme will build up a "strong and dedicated guild" of scientists devoted to the clean-up problem.

The programme, says Grumbly, will give both the US and the international research community the opportunity to participate in "closing [a] circle" which began with the development of the atomic bomb. Scientists outside the United States are, at least in theory, eligible to apply for the grants.

Grants will be available to investigators in chemistry, biology, geophysics and any other branches of science with potential relevance to the clean-up of the sprawling weapons complex. Problems to be dealt with include the characterization of nuclear waste, the *in situ* treatment of buried waste, the behaviour of plutonium and the implications of treatment on ecosystems.

An invitation for grant proposals published in the *Federal Register* on 9 February specifies that brief pre-applications need to be received by 28 February and full proposals by 8 May.

The programme is starting in a hurry — and a year earlier than the DoE had been planning — because John Myers (Republican, Indiana), chair of the House of Representatives appropriations subcommittee with jurisdiction over the department, forced the inclusion of the money in this year's appropriations bill.

Grumbly has long argued that scientific research should lead the clean-up process. Until now, however, no money has been devoted directly to it. The OEM spends \$80 million a year with universities, he says, but that money is intended to develop

existing clean-up technologies.

The Galvin Commission, which issued a scathing assessment of DoE laboratories last February (see *Nature* 373, 463; 1995), suggested that an annual \$400 million of the OEM's \$7 billion budget be transferred immediately to science and technology, rising to \$800 million later.

But the department is under constant pressure to produce immediate clean-up results, while keeping as many people as possible employed on the now-redundant nuclear weapons sites; 13,000 people, for example, work at the most heavily contaminated site at Hanford in Washington state.

Galvin also criticized the lack of scientific



expertise in Grumbly's office. The new programme will therefore be run in partnership with the DoE's Office of Energy Research (OER), which will help to organize the external peer review of grant applications.

Michelle Broido of OER says that grant applications from both universities and DoE laboratory applicants will be peer reviewed "subject to the same criteria". But, under US law, the laboratories cannot compete with universities for the same money, and \$20 million will therefore be set aside for each group.

Grumbly says that the programme will "develop a community of scientists who will bridge the gap" between environmental scientists in the universities, who have traditionally been funded by the Environmental Protection Agency (EPA), and the nuclear scientists and engineers in the weapons complex who have recently turned their attention to the clean-up problem.

He adds that the science programme might take "8-10 years" to produce results applicable to clean-up. The National Academy of Sciences will be asked to evaluate the programme.

Colin Macilwain

HIV vaccine strategy seeks to woo industry

Washington. The US National Institute of Allergy and Infectious Diseases (NIAID) has announced a new strategy for HIV vaccine development aimed at strengthening government-industry collaboration and countering market obstacles that have driven companies from this high-risk field.

For the first time, government scientists will specify in advance the scientific criteria to be met before a potential vaccine proceeds to the next stage of development. This essentially guarantees to companies that they will be able to proceed step-by-step towards licensing if they meet specific requirements.

Anthony Fauci, the director of NIAID, outlined the new plan at a conference on AIDS Vaccine Development held last week at the National Institutes of Health (NIH), of which NIAID is a part.

The plan stresses four points. First, the marriage of fundamental to empirical research, to achieve what Fauci calls a "golden mean" of balance between the two. Second, the development of more and better-defined industry-academic collaboration, without which Fauci said the development of a successful vaccine will be "virtually impossible". Third, the better exploitation of opportunities to speed vac-

cine research. Finally, enhanced links between NIAID and public and private organizations in the United States and elsewhere, including the United Nations AIDS Programme, Britain's Medical Research Council and France's Agence Nationale de Recherches sur le SIDA.

Fauci later described it as "critical" that the NIAID, in dealing with industry partners, should agree with such partners in advance the scientific criteria to be met before a vaccine proceeds to the next step towards licensing. Prospective agreement on the criteria — which will be determined on a case-by-case basis — will result in a "level playing field" ensuring that "we don't change the rules of the game midway through", said Fauci.

The announcement pleased vaccine developers. "There will be some level of clarity about how these decisions will be made at the start," says Dino Dina, president of Chiron Biocine, the vaccine division of biotechnology firm Chiron, based in Emeryville, California. "There will not be an open-ended set of goals."

In the past, US government scientists have not used pre-agreed criteria in deciding whether to allow vaccines to proceed to the next step in the multistage process of

vaccine development leading from the laboratory to animal testing, and — in theory — to eventual large-scale tests in humans.

But the latter have not yet taken place. Some in industry claim that companies have been discouraged by a perceived arbitrariness in federal decision-making, leading to a recent decline in the number of companies involved in vaccine development (see *Nature* 378, 323; 1995).

Precise criteria to be applied under the new plan have not been established for any potential vaccine. But these could include factors such as the requirement that a minimum percentage of volunteers develop defined levels of neutralizing antibodies or cytotoxic lymphocytes before a vaccine can proceed to the next stage.

Fauci remains optimistic that an effective, safe vaccine can be developed, despite obstacles such as the ability of the virus to mutate rapidly. But he says that it is "inappropriate" for those watching vaccine development to focus solely on achieving immunity to HIV. Researchers may well be able to influence the AIDS epidemic dramatically merely by helping the immune system to fight the virus, thereby lowering its concentration in the blood, he said last week.

Meredith Wadman

Yeltsin promises to pay government's debts to scientists

Moscow. Boris Yeltsin, the Russian president who is currently engaged in a bitter re-election campaign, has responded to protests from scientists about the continued funding crisis in Russian science by promising to clear the government's debts to researchers by next Sunday (25 February).

Delivering a speech at a ceremony for the state science and technology awards earlier this month, Yeltsin said he was aware of the hardships facing the scientific community and announced that 50 billion rubles (US\$1.05 million) had been allocated to clear unpaid salaries and outstanding laboratory maintenance costs.

Yeltsin added that he was aware of the dwindling prestige of science in Russia, and said that, as a result of urgent measures being taken to redress the situation, scientists would soon feel their plight somewhat eased. "The time will come when scientists from all over the world will be happy to come to work in Russia," he promised.

Finance for Russian science has been reduced 15-fold since 1990. But for the past several months, money for science — which now amounts to 0.32 per cent of gross domestic product — has not been released by the government. As a result, institutes of the Academy of Sciences have been unable to pay salaries or electricity and gas bills.

At the same time, it is unclear whether

Yeltsin's promise will be sufficient to end the protests. Last week, prominent members of the academy threatened mass hunger strikes and the picketing of government buildings unless the government allocated 250 billion rubles to settle arrears owed to them. They also observed a 'day of action' in protest at the financial freeze.

Furthermore, many believe that Yeltsin's decision to announce the release of funds

reality Council, Yeltsin spoke of the need to maintain Russian technological independence. In a throwback to old Soviet rhetoric, he lamented the "foreign threat to the state, caused by the revealing of our technological secrets" and of "foreign intelligence activity in the field of Russian scientific and technological achievements", language disturbingly similar to that used earlier to criticize the work of foreign-funded scientific bodies.

Some observers also see evidence of a resurgence of old Soviet ideas in moves to establish a new and generously state-financed Russian Academy of Technological Sciences (RATS), aimed at coordinating the efforts of scientists and engineers working in the defence industry.

On 7 February, Oleg Soskovets, the prime minister's first deputy, issued a directive for work to begin on setting up the RATS. Its creation was initially rejected by the Russian Duma just over a year ago (see *Nature* 372, 208; 1994). But an organizing committee headed by Evgeny Velikhov, vice president of the Russian Academy of Sciences, has now been charged with preparing relevant documents, including a draft decree to be signed by Yeltsin.

The 400 scientists and engineers elected to the new academy will be entitled to life-long stipends equal to members of the Russian Academy of Sciences.

Carl Levitin

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Yeltsin: claims awareness of hardships.

for scientists, and earlier announcements supporting the need for research, may also be tied to political motives. Russians have been hit hard by economic reforms, and many hanker after the old days when jobs and food — though rationed — were more readily available than they are today.

At a recent meeting of the Russian Secu-

South Africa boosts funding for universities

Cape Town. The South African government has announced that it plans to increase spending on universities and 'technikons' — higher technical institutes — by more than 23 per cent next year. This represents an increase of R830 million (US\$231 million) in its support for higher education.

The move follows the determination of the coalition government led by the African National Congress, and expressed through the efforts of the Minister of Education, Sibusiso Bengu, to stop the downward slide in subsidies that has taken place over the past decade. Bengu, a former rector of the University of Fort Hare, is acutely aware of the problems that this has posed for the universities.

But the increase will not be uniform. Universities will receive 20.9 per cent more than last year, while technikons will receive an additional 31 per cent. This reflects the fact that the funding system has in the past been skewed in favour of universities, as well as the government's determination to encourage the acquisition of technical skills among school-leavers.

As the full budget will be announced only in March, it is not yet clear where the government is cutting back to make more funds available for tertiary education. In the past, tertiary education has received 15 per cent

of the education budget, and the increases may either reflect an overall increase in the education budget, or may be at the expense of state expenditure on schools.

As previously, grants to individual institutions will be made according to the so-called 'SAPSE' formula. This is based primarily on levels of student enrolment and success — with different weighting for science and arts courses and undergraduates as opposed to postgraduates — but also incorporates research output.

To decide the actual subsidy received, the score registered by an individual institution is multiplied by a so-called 'A factor', which represents the proportion of its entitlement that the state can afford to finance. This has been increased from 62.8 to 66.2 per cent for universities, and 61.1 to 68.2 per cent for technikons.

Both types of institutions are expected to make up the balance in operating costs from student fees. But the cumulative effect of rising fees over the past decade has led to serious payment problems, particularly at universities with large numbers of black students. Earlier this month, for example, the University of the Western Cape (UWC) was forced to close temporarily following disruptions that had resulted from the university's refusal to register students who had

not paid last year's fees.

The increased subsidy will affect institutions in different ways, primarily reflecting different levels of increase in enrolment in 1994 (subsidies are calculated on the basis of figures two years before their payment). UWC will benefit the most, with an increase in its subsidy of 49.39 per cent.

In contrast, the University of the Witwatersrand will receive an increase of only 8.8 per cent, while two of the former 'homeland' universities of Transkei and Bophuthatswana will suffer cuts of between 6 and 7 per cent. This is because their 'A factors', which were significantly higher than the other universities over the past few years as a consequence of receiving special treatment from the previous government, have now been brought more into line.

In addition to the 'A factor' increase, the part of the formula corresponding to academic and administrative staff salaries has also been increased. Hugh Amooore, registrar of the University of Cape Town, says that the government's recognition that academic salaries are lagging is important, but that the funding of either bursaries or loans for indigent students has still not been adequately addressed.

The increases have been welcomed by Andre de Wet, registrar of finance at UWC. "Now we can look at putting some more staff in the lecture halls as well as improving our laboratories and libraries," he said.

As individual institutions have the autonomy to manage their own finances, universities may also choose to respond to the increases by limiting fee rises next year, by directing more funds into their own loan or bursary schemes, and by raising salaries by different amounts.

The National Commission on Higher Education, appointed more than a year ago under the chairmanship of Jayairam Reddy, former rector of the University of Durban-Westville, is due to present its final report to government by the end of July. Its brief is to completely revamp the funding system for tertiary education. This follows widespread criticism that the SAPSE formula does not relate to South Africa's graduate needs, as it does not dictate the number of places that the state subsidizes in each discipline.

Despite weightings in favour of students registered in the natural sciences in terms of the formula, this has led to a situation in which the proportion of university graduates in science and engineering fell from 17 to 13 per cent of the total, and those in medicine from 11 to only seven per cent, between 1982 and 1992. An additional possibility being considered by the commission is the amalgamation of the universities and technikons into a single sector, as has recently taken place in the United Kingdom.

Michael Cherry

Funding agency names new president

Cape Town. The South African Foundation for Research Development (FRD) announced last week that Khotso Mokhele, one of its two vice-presidents, will become its new president from 1 April. Mokhele, who will take over the post on the retirement of Rein Arndt, will be the first black person to head a science council in South Africa.

Mokhele becomes head of the government's principal funding agency for science and engineering research at a time of growing concern about research funding in South Africa. "The FRD is R30 million (US\$ 8 million) short of what it requires to implement its research programmes this year," he says. "A critical task will be to mobilize these funds."

He admits that an even more important challenge will be to change the demography of the science and technology community in South Africa, in terms of both race and sex; 30 per cent of scientists working in South Africa are women, but only 15 per cent are not white. In engineering all but five per cent are white, with only 1.6 per cent of engineers being female. "But at the same time we shall have to ensure that the quality of both our research and our

graduate students is not only maintained but enhanced."

Born in Bloemfontein, Mokhele, who is 40, is a microbiologist who studied as



Mokhele: first black to head science council.

an undergraduate at the University of Fort Hare in South Africa's Eastern Cape Province. He then completed master's and doctoral degrees at the University of California at Davis, the latter under the former president of the American Society of Microbiologists, John Ingraham.

After spending a year as a postdoctoral fellow in the laboratory of the Nobel prizewinner Hamilton Smith at Johns Hopkins University in Baltimore, Maryland, Mokhele returned to a faculty position at the University of Fort Hare for three years. He then moved to the University of Cape Town in 1990, before being appointed to his current position at the FRD in 1992.

M. C.

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ESA/ISO, CEA Saclay and ISOCAM Consortium

Cold spots: ISO's view of Whirlpool galaxy.

Infrared images map 'invisible' matter

Paris. The European Space Agency (ESA) last week released the first data from its Infrared Space Observatory (ISO), which was launched last November and is intended to reach the cool 'hidden' parts of the Universe other types of telescope cannot reach.

The ISO image (above) of the Whirlpool galaxy NGC 5194/5, for example, 20 million light years from Earth, shows bright spots in the spiral arms corresponding to warm dust clouds where stars are being formed and areas of cooler dust among and between the arms that represent the legacy of debris left by previous generations of stars.

"I'm absolutely delighted [with the first data]", says Catherine Cesarsky, the principle investigator for the telescope's camera. She points out that ISO marks an advance on previous infrared instruments — such as the joint UK/Dutch/US Infrared Astronomical Satellite, IRAS, which mapped 250,000 cosmic infrared sources in 1983 — in that it has better resolution and can measure radiation over the whole infrared spectrum. "We only had a rough picture before," says Cesarsky. "Now we can really do physics."

In particular, ISO's high sensitivity will allow astrophysicists to study the birth of stars and solar systems, while its ability to see cold 'invisible' matter will also help in the search for the Universe's 'missing mass' — the discrepancy between the mass of the Universe as estimated from gravitational calculations and that estimated from what can be seen in visible light.

ISO's successful entry into operation also makes Europe the apparent winner of a race between ESA and the US National Aeronautics and Space Administration (NASA) to fly a high performance infrared telescope, according to Cesarsky. She points out that although NASA began work on a similar telescope, SERTIF, before ESA, it has yet to complete the project.

The United States, she says, has now adopted an attitude of "if you can't beat them, join them". Indeed, both the United States and Japan have now reached agreement with ESA for the use of ISO. **D. B.**

French biomedical research 'needs more coordination'

Paris. France's secretary of state for research, François d'Aubert, has called for greater collaboration between those carrying out fundamental biomedical research and clinical researchers. This was one of three proposals contained in the government's first policy statement on research since d'Aubert took office last November, which was jointly presented to the cabinet last week by d'Aubert and Hervé Gaymard, secretary of state for health and social security.

The statement points out that although fundamental biomedical research is internationally competitive, France is relatively weak in clinical research and biotechnology. In particular, d'Aubert pointed out that only one French drug company ranks among the world's ten largest pharmaceutical groups, that no French-produced drug ranks among the top twenty best-selling drugs, and that the United Kingdom, for example, has four times as many biotechnology companies.

Closer collaboration between fundamental research and clinical research is one means to remedy this situation, according to d'Aubert, who called for increased research training in medical schools, greater mobility among clinical and basic researchers, and a reinforcement of existing joint structures

between hospitals and public research bodies such as university teaching hospitals.

But observers are sceptical that these proposals amount to much more than good intentions and feel they are unlikely to lead to many concrete changes. "I am not taking this statement at all seriously," says one official from the national biomedical research agency, INSERM.

Indeed, d'Aubert's call for greater collaboration between clinical and basic research is seen by some as partly a reflection of the research ministry's desire to increase its control over the FF300 million (US\$59 million) that the ministry of health takes from the hospital budget annually for clinical research. "If there is an urgent reform needed", quips one such critic, "it is for better cooperation between the ministries of health and research".

Research officials are nonetheless pleased with one aspect of the government's statement, its enthusiastic endorsement of the value of the so-called Instituts Fédératifs de Recherche (IFR). These are loose federations of laboratories that bring together scientists from research centres, universities and hospitals to create a critical mass in particular research areas at a single site.

Declan Butler

Montagnier sets up AIDS research centre

Paris. An 'Integrated Centre for Clinical and Biomedical AIDS Research' was this week formally inaugurated in the grounds of St Joseph's Hospital in Paris. It has been founded by Luc Montagnier of the Institut Pasteur and a member of the French group that discovered the human immunodeficiency virus (HIV) in the early 1980s.

The centre — whose FF18 million (US\$3.6 million) construction costs were collected as part of a television campaign to raise funds for AIDS research — will have about 50 staff members and will bring together clinical and basic researchers at the same site. Its FF8-million annual running costs will be met partly by the national AIDS research agency (ANRS) and further fundraising rounds, Montagnier says.

The centre's main clinical activity will be to study the association of various antivirals at a very early stage of infection by HIV. Whereas patients are usually treated only when their CD-4 levels fall below 200 cells per microlitre, the centre will treat patients with CD-4 levels greater than 500. The idea, says Montagnier, is to find out whether such treatment can delay the onset of AIDS, for example by reducing viral load.

Such an approach raises the ethical

problem of treating otherwise healthy patients with drugs that generally have side-effects, admits Montagnier, who says all patients will be asked to give their informed consent to treatment. But he insists that the centre's main goal will be patient care. "It will be research in the service of the patient", says Montagnier, who argues that clinical research traditionally uses "patients in the service of research".

To evaluate the outcome of such early treatment, the centre will carry out detailed follow-up of individual patients using a variety of markers designed to measure, for example, cell death, the level of activation of the immune system, oxidative stress and cofactors — Montagnier has long argued that cofactors such as mycoplasmas increase the pathogenicity of AIDS. A "battery" of such markers is needed, he says, to establish which phenomena may be important in disease progression.

Basic research at the centre will focus on immunological aspects of the control of viral replication. This includes factors such as the role of oxidative stress in the development of the disease, and the mechanisms of protection among seropositive long-term 'survivors'. **D. B.**

Votes nudge Australia closer to joining telescope project

Sydney. Australia's general election, which takes place next week (2 March), may have helped to provide the country's astronomers with an entry ticket to the world's largest optical and infrared telescope, currently under construction in Chile as part of the European Southern Observatory (ESO).

Last December, Australian astronomers were shocked when the government announced that it was not prepared to include a contribution of A\$28 million (US\$21 million) over six years to the ESO in a round of major national research facilities announced as part of a broad innovation statement. Seven other projects worth \$A62.4 million over six years were supported (see *Nature* 378, 653; 1995).

Last week, however, in the middle of an election campaign in which the Labor government's popularity has been trailing that of the conservative coalition of the Liberal and National parties, Peter Cook, the Australian science minister, announced that Australia's membership of the ESO will indeed be a priority within Labor's next budget in August, if re-elected, with increased funding of A\$30 million over a shorter time-scale of five years.

The ESO project has been under preparation for two years since Australia became the ninth and only non-European nation to be invited to join the consortium. The rejection of funding in December put continuity of the invitation in serious danger.

Australian participation had already received unanimous endorsement from the astronomy community and, despite costing more than other applications for funds, had received top ranking from two expert committees set up by the government.

Furthermore, Australian expertise in optical interferometry is considered especially valuable for the Very Large Telescope (VLT), with its four 8.2-metre mirrors, which is now being built at Cerro Tololo on Mount Paranal in Chile.

If ESO's invitation is accepted, then Australia can expect trade-offs for its contribution in terms of design and construction contracts. At the same time, Australian astronomers have been anxious to retain eminence in Southern Hemisphere astronomy through a share in the VLT, which will be at a scale and altitude higher than possible on their own continent.

Cook has not given any public explanation

of why the recommendations of the expert committees were apparently ignored by the government in December. But a spokesman said the government had been reluctant to spend such a large proportion of the facilities fund on a single project, or on one based overseas.

In the lead-up to the election, however, the minister appears to have been embarrassed by adverse public comment on the December announcement. Within two days, he was having meetings with astronomers, promising them that he would seek extra funds to resuscitate the ESO plan.

Negotiations with ESO took place early in February, when Jeremy Mould, director of the Mount Stromlo and Siding Spring Observatories, and Michael Pitman, the government's chief scientist, visited ESO headquarters at Garching in Germany. They returned to Australia with a draft agreement two days before Cook's speech.

Cook has now exchanged letters with ESO, and has set up a four-man group to negotiate the details, as well as making a public commitment to funding. He also promised to contact his opposite number, Robert Hill, to seek the coalition's agreement to making the ESO deal a bipartisan policy.

Astronomers have also been keeping their lines open to Hill, given the possibility of a change of government in the elections. Hill confirms that under a coalition government, the negotiations on Australian participation in the project would continue.

Ironically, the very international aspects that had worked against the ESO link last year are now counting in its favour. Cook said last week that he wants to back more new projects, including a centre of excellence in radioastronomy to serve the Asia-Pacific region, in which Australia is seeking a leading economic and political role, and an infrared telescope in Antarctica.

The December decision reflected lack of interest among Australian politicians in supporting European links at a time when they saw the future of trade and links in science and technology as lying in the Asia-Pacific region. But the route to wider opportunities for Australian science is now back in favour.

But a shadow still hangs over the announcement. No new agreements can be concluded by the government as caretaker during an election campaign, after which the government or minister may change. No matter what the outcome of the election, the country's economic difficulties could still provide whichever party gains power with justification for slashing expenditure.

Peter Pockley

Review panel cancels meeting as gene therapy proposals fall

Washington. What had previously been a regular stream of proposals for innovative human gene therapy experiments has dried up, prompting an advisory panel of the US National Institutes of Health (NIH) to cancel its next quarterly meeting.

But members of the Recombinant DNA Advisory Committee (RAC) deny the drop is related to a highly critical report on gene therapy commissioned by Harold Varmus, the director of NIH, and delivered to him in December (see *Nature* 378, 655; 1995).

"It's too early to talk about a trend," says LeRoy Walters, acting director of the Kennedy Institute of Ethics at Georgetown University in Washington DC, and acting chairman of the committee. "We could find that [the] number of protocols submitted rebounds."

The report, prepared by a panel chaired by Stuart Orkin of Harvard Medical School and Arno Motulsky of the University of Washington, warned researchers against "overselling" gene therapy, and called for more emphasis on basic research.

Walters conceded that the need for researchers to "read and discuss" the report may have slightly dampened applications to the 25-member RAC, which has cancelled its March meeting on the grounds that it does not have any protocols to review. But he suggests that heavy snowfalls, US government shutdowns and the holiday season are likely to have played larger roles.

Nelson Wivel, executive secretary of the RAC, says that a new, streamlined application procedure has also contributed to a mistaken perception that gene therapy applications are down. Since July 1995, gene therapy protocols that are not substantially original are forwarded directly to the Food and Drug Administration, bypassing the RAC.

What has plummeted, says Wivel, is not the total number of applications but the number being subjected to full RAC review. For example, at its December quarterly meeting, the RAC reviewed just one original protocol, namely one for treating ornithine transcarbamoylase deficiency, a rare genetic disorder involving urea metabolism.

Some researchers say that the decline in original protocols is not surprising because the limits of current technology are being reached. "It's exactly what you'd expect," said Malcolm Brenner, director of the cell and gene therapy programme at St. Jude's Children's Research Hospital in Memphis, Tennessee. "As certain technology becomes available, it gets developed to a certain level. Then there's a pause while everybody moves on to the next level. That's beginning to happen."

Meredith Wadman



Cook: accepts ESO should be a priority.

German institutes face closure after review

Munich. The future of some of Germany's 82 'blue-list' institutes may be in doubt as a result of a review of the research activities of all such institutes by the Wissenschaftsrat, Germany's influential science council.

Blue-list institutes form the 'fourth pillar' of Germany's non-university research, after the Max Planck Society (MPG), the Fraunhofer Society and the national research centres. Funded jointly by federal and *Länder* governments, they carry out a variety of research and service activities in the social and natural sciences.

After reunification, many former East German establishments were added to the 'blue list' as a stopgap measure, as neither the MPG nor the universities were able to absorb them. Largely as a result, the number of such institutes has risen from 48 to 82.

The body responsible for the institutes, Wissenschaftsgemeinschaft Blaue List (WBL) was reorganized in 1993 to take this into account. But its increased significance has come in for some sharp criticism (see *Nature* 363, 482; 1993).

As a result, the Bund-Länder-Kommission (BLK), which arranges political and financial agreements between federal and *Länder* governments, decided to implement a review of the activities carried out under the umbrella of the WBL, likely to last for five years, as a concluding chapter to its re-unification efforts.

Following the first stage of this review, in which five blue-list establishments were assessed, the Wissenschaftsrat has now recommended that funding be withdrawn from two of them. According to the science

council, both the Institute for Economic Research in Hamburg and the Department of Earth Sciences at the Office of Soil Research (NLF-B-GGA) in Lower Saxony, have been performing badly in terms of publication, cooperation with universities and general efficiency.

The Wissenschaftsrat had already recommended to the BLK, which is responsible for the final decision, that funding be withdrawn from the Institute for Mineral Oil Research (IfE) in Lower Saxony.

The Wissenschaftsrat has said that it does not doubt the "general importance" of the work of these institutes. But their future now looks bleak, as termination of their contracts with the WBL would mean funding running out by the end of 1997. At a time of tight research budgets, neither their host *Land* nor a federal ministry is likely to take on the costs of running institutes that carry out a relatively low level of research, and as a result, the more productive research projects could end up being taken over by university departments.

Neither the BLK nor the affected institute directors are willing to discuss the possibility of closure. Dagobert Kessel, head of the IfE, says confidently that "there will be some kind of mineral oil research even in the year 2000", while Ralph Hämel, head of NLF-B-GGA, complains of the "thoughtless assessment" of the Wissenschaftsrat, and says that he is planning to object to its conclusions.

But Michael Lankeit, administrative vice president of the WBL, argues that the Wissenschaftsrat's assessments are fair. "My colleagues must finally recognize that the Wissenschaftsrat is serious about it," he says.

Lankeit is aware that the Wissenschaftsrat operates under political and financial pressure. Although the budget of the WBL is being held constant, eight new institutes have been recommended for admission by the Wissenschaftsrat. As 16 establishments are now to be assessed every year, he warns all WBL institutes against a complacent "why should it happen to us" attitude.

Having asked the Wissenschaftsrat to carry out the evaluation, the BLK is expecting results, says Lankeit, as part of its efforts to consolidate Germany's system of research funding and to boost quality control mechanisms that have been strained by the demands of reunification and the economic recession (see *Nature* 377, 466; 1995).

At the same time, however, when the WBL agreed to the evaluation of its institutes, it did so in the hope that it will eventually be given similar control over its finances to MPG and the Fraunhofer Society. Until now, the WBL has not been able to reallocate any savings in its budget, but has been required to pass these back to the Bund and the host *Land*.

Quirin Schiermeier

Combined beams bring stars into focus

London. A new type of optical telescope, designed and built at the Mullard Radio Astronomy Observatory at the University of Cambridge and costing less than one thousandth the price of the orbiting Hubble Space Telescope (HST), has produced the sharpest ever images of Capella (below), two closely spaced stars 40 light years distant from Earth.

No other telescope, including the HST, has been able to resolve these stars, which are separated by a distance less than that between the Earth and the Sun. But the Cambridge Optical Aperture Synthesis Telescope (COAST), which is pictured right, cost only £850,000 (US\$1.3 million).

The telescope will be able to discern detail in stars in a manner equivalent to resolving "the letters of a car

number plate from a distance of 1,000 km", says Peter Warner, assistant director of research at the Mullard Laboratory.

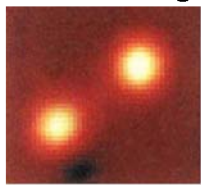
The telescope is based on interferometry. It combines light beams from an array of up to four smaller telescopes, each pointing towards the chosen star, and the desired image is then reconstructed electronically from the recombined beams. This technique is widely used in radioastronomy. Until now, however, it could not be used at the much shorter optical wavelengths, where a higher degree of precision and

stability is needed to recombine the separate beams accurately.

The challenge for COAST's designer, John Baldwin, professor of physics at the Mullard Laboratory, and his team was to produce a design that would allow for thermal expansion in the telescope's parts, possible ground movement and temperature changes.

The beam from each telescope passes through a plastic tube, which isolates it from the atmosphere, and enters a 'beam-combining building', a 32-metre-long corrugated tunnel 2.4 metres high. Stability is provided by submerging the tunnel in 300 tonnes of earth covered with grass.

Baldwin and his team also had to



maintain the beam's path length, which would otherwise be altered by the Earth's rotation and the star's movement. This was done by allowing light to fall on to a mirror mounted on a movable but frictionless trolley. A laser tracks the minute changes in the beam's path, and the trolley's position is altered accordingly.

COAST was paid for by the Particle Physics and Astronomy Research Council. The designers at Mullard are now hoping to build a second version which they expect to have a 10-fold better resolution

Ehsan Masood

US funding announced for studies of genome patenting problems

Washington. The US Department of Energy, one of the main sources of funding for the Human Genome Project (HGP), has announced that it is making available up to \$1.3 million next year for studies of the intellectual property issues associated with the project.

The announcement comes as lawyers, academic scientists and industry officials are having to acknowledge that there are no easy answers to the explosion of complex intellectual property issues raised by modern molecular biology. "These are difficult problems that defy facile solutions," Rebecca Eisenberg, a patent law professor from the University of Michigan Law School, said last week at a forum on intellectual property and research tools in molecular biology, held at the National Academy of Sciences in Washington.

Lita Nelsen, a technology transfer administrator at the Massachusetts Institute of Technology, said that current practice required "trading off" the often competing interests of industry, government and universities. The latter, she said, are, as a result, "trying to evolve norms based on this balancing of equities".

The forum focused primarily on the impact of patenting research tools on their dissemination and use. In this, Eisenberg argued, even segments within industry — for example, small biotechnology companies as against large pharmaceutical companies — can have fundamentally different interests. □

Germany simplifies biotech rules

Munich. The German cabinet has made a number of changes to genetic engineering regulations to make work with genetically modified organisms easier. The formal procedures authorizing work with genetically-altered plants have been tightened up, and the amount of documentation required has been reduced.

The German health ministry says that European legislation is

becoming more important than national legislation. To protect research in Europe from suffering in competition with Japan and the United States, European rules on biotechnology should be revised. The ministry has expressed its appreciation of a decision last December by the European Commission to amend directives on the use of genetically manipulated micro-organisms in closed systems. □

Capital boost for French stocks

Paris. The French stock exchange last week opened a new stock market, the *nouveau marché*, modelled on the US NASDAQ, and designed both to make it easier for young high-technology companies throughout Europe to raise capital and to provide venture capitalists with the opportunity to recover their investments by floating companies they have supported.

The lack of such possibilities has long been considered to be one cause of Europe's poor record in creating start-up companies. François d'Aubert, the French secretary of state for research, last week described the *nouveau marché* as "indispensable", predicting it would encourage researchers to create their own companies. □

India calls for nuclear weapons ban

London. India has called for an international convention that would prohibit the use or threatened use of nuclear weapons by any nation. The proposal, put forward by Arundhati Ghose, India's representative to the international disarmament conference in Geneva, follows an earlier suggestion by India that the Comprehensive Test Ban Treaty (CTBT) be tied to a promise by the five official nuclear powers to dismantle their existing nuclear weapons according to an agreed timetable.

India is believed to have the technology to manufacture nuclear weapons. It carried out a nuclear test in 1974, but insists that it was a 'peaceful' explosion, and that it does not officially possess a nuclear bomb. The government has refused to sign the Nuclear Non-Proliferation Treaty, partly on the grounds of a potential nuclear threat

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Genes, patents and insurance

SIR — The recent Commentary on “The case for genomic patenting”¹ makes three questionable points in attempting to justify the corporate interests of Human Genome Sciences (HGS) and SmithKline Beecham Pharmaceuticals (SKB) with regard to their expressed sequence tag (EST) database, while at the same time criticizing the position of SKB’s rival, Merck & Co.

These points are (1) that ESTs should be patentable, (2) that the HGS conditions for access to its EST database are reasonable and (3) that Merck lacks an “intellectually consistent position” in attempting to create a public domain EST database.

On the first point, the author glosses over compelling arguments, as previously made by others², that ESTs are not patentable because they lack utility. ESTs lack utility for patent purposes when the function and coding sequence of the gene represented by the EST are unknown. The argument that an EST has utility as a diagnostic test is speculative without an actual demonstration of such a test. The policy argument that ESTs should be patentable because publication of an EST prevents a later patent on the entire sequence is not consistent with established legal precedent. In the United States, at least, it is well established³, that in order for a new compound (that is, the full-length sequence) to be considered obvious over a related compound (the EST) there must be some motivation to prepare the new compound. Without any information as to the actual biological function of the full-length sequence, there is no motivation to prepare it from the EST.

On the second point, the author states that the HGS-SB terms for database access are no more onerous than the National Institutes of Health’s Uniform Biological Material Transfer Agreement (UBMTA)⁴. The past requirements of HGS for a mandatory exclusive licence to inventions made from the database have apparently been relaxed. HGS now requires “only” an option to negotiate an exclusive licence. This is a recent — and welcome — change brought about in part by public controversy⁵ and the refusal of many academic institutions to accept the earlier terms⁶.

The terms of the new option should not be compared to the UBMTA, however, because the HGS terms apply in cases where only information — not materials — is received from HGS. Furthermore, they apply to all inventions produced as a result of the recipient’s use of the HGS data/material. The only intellectual property right flowing back to the provider under the UBMTA applies to inventions made by the recipient that are termed

“modifications”⁷. “Modifications” are defined in the UBMTA as materials made by the recipient that physically incorporate the provided material. The recipient is free to patent and license inventions made using the provided materials, even as to modifications, so long as the modifications are not physically transferred to the licensee and the recipient notifies the provider of the filing of the patent application⁸. These patent terms are less burdensome on the recipient university than even the current HGS terms.

On the third point, Merck’s efforts⁹ should be applauded. Ultimately, the entire human genome will be sequenced, perhaps as early as 2001 (ref. 10). Hopefully, full-length sequence information will be publicly available, and the present debate will become essentially moot, as has the earlier controversy about patenting genetic markers¹¹.

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SIR — Following your recent article about patenting of human genes (*Nature* **378**, 756; 1995), I should like to clarify the position of Compassion in World Farming (CIWF) on patenting.

CIWF is totally opposed to the patenting of transgenic animals. We fear that the availability of patents will give a huge commercial boost to genetic engineering, a process that poses very considerable threats to the health and welfare of farm animals. The risk of suffering is present both in the laboratory at the development stage and then on farm for the newly ‘created’ animals.

There is a growing belief that a responsible society should treat animals not as being placed in this world for our convenience but as living creatures capable of feeling pain and suffering. Patenting, involving as it does regarding animals as inventions, as things, is out of step with this modern approach to animals.

If, despite our firm opposition, the proposed European Union directive does eventually sanction the patenting of animals, it is vital that European patent

law should provide some real protection for the animals involved.

Peter Stevenson

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SIR — I was delighted to see that your first Briefing section contained a full discussion of the implications of the increasing amount of genetic information for the insurance industry (*Nature* **379**, 389–392; 1996). This was one of the concerns discussed in the Science and Technology Committee’s report on *Human Genetics; The Science and its Consequences* (HC (1994-95)41-I).

I fear, however, that you misrepresent the committee in saying that the report supports Dr Nicholas Barr’s proposals for spreading risk; as we commented, “such a scheme would need detailed study and design”. We did not offer any further opinion on its merits. The significance of the scheme proposed by Barr, and another suggested by Professor Kenneth Arrow, is that they suggest that the problems are not insuperable and that “it would be possible to find ways to regulate the use of genetic information in insurance which would both protect the interests of society in enabling as many people as possible to obtain insurance and protect the insurance companies themselves”.

We hope the insurance companies will themselves produce such a solution.

Giles Shaw

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Art for science’s sake

SIR — In the discussion about the prevalent distrust of science, it seems to me important that scientists should continue to be open to the influence of art and poetry and other aspects of culture, as they were previously. We don’t have to love avant-garde art, but we should show our cultural interests in discussions with students, for example. And poets and other artists should be able to integrate scientific concepts into their art.

I also believe that scientists should be more willing to admit that in spite of their achievements, there is still a great deal to be known about the world around us.

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Diversity and sustainability on the prairie

Peter Kareiva

The idea that higher biological diversity begets more robust and more productive ecosystems is an old one. Only now are convincing experimental tests of the principle emerging.

PAUL and Anne Ehrlich produced one of the environmental movement's most compelling analogies when they likened the extinction of species to the "popping of rivets" from an aeroplane¹. The Ehrlichs' vision of an aeroplane gradually weakened by the loss of too many seemingly inconsequential rivets makes vivid the possible risks of relentless extinction. Unfortunately, getting beyond the rivet analogy to concrete evidence for the 'function' of biodiversity has been a very difficult task. On page 718 of this issue², Tilman, Wedin and Knops make a major advance by providing striking experimental evidence that biodiversity in Minnesota grasslands increases sustainability and productivity.

The experiment is the latest of a handful of exciting studies pointing towards an ecological 'value' for biodiversity. For instance, Tilman's group previously published an analysis suggesting that species-rich grasslands are more resistant to the ravages of drought and recover more rapidly from drought than do species-poor grasslands³. But this earlier finding was not the result of an experiment that was intended to uncover the consequences of biodiversity — instead the correlation between grassland diversity and stability emerged serendipitously from a study aimed at unveiling the effects of nitrogen on plant competition.

Among the few experiments explicitly designed to assess the function of biodiversity, only a microcosm experiment⁴, performed at the Centre for Population Biology's Ecotron facility at Silwood Park, Berkshire, rivals the clarity of the new work from Tilman *et al.* In the Ecotron experiment, it was found that a particular diverse food web involving annual plants and their associated insects and soil fauna was more productive than species-poor communities that were subsets of the diverse assemblage. But whereas the Ecotron results are prone to the criticism of contrived simplicity, and the fact that they reflect events in only a single diverse community, Tilman and colleagues' grassland experiment circumvents these limitations through clever experimental design and herculean field work.

Building on the Ecotron results and Charles Darwin's hypothesis that more diverse plant communities are more productive (see box overleaf), Tilman *et al.*

directly manipulated plant biodiversity and measured plant production and the effectiveness with which plant communities representing different biodiversities extract nitrogen from the soil. Specifically, 147 plots, each three metres by three metres, of comparable soils and nitrogen content were established. These plots were sown with the seeds of 1, 2, 4, 6, 8, 12 or 24 species of native North American prairie plant species, with a minimum of

could be clearly seen in the form of increased productivity on the diverse plots (measured by percentage cover). Not only was this pattern evident in the experimental plots, but it could also be found in a sample of 30 different unmanipulated native prairies. The presumed mechanism for these results is that, in more diversified communities, interspecific differences in soil use allow fuller use of nitrogen, the main limiting nutrient.



Plots of difference — a view of Tilman and colleagues' biodiversity experiments.

20 replicates for each diversity treatment (see photograph). The elegant aspect of the experimental design was the fact that exactly which species were sown into each plot was a random draw from a pool of 24 native species — meaning that results obtained for the single-species treatment were not due to a particular species but to 20 random draws of single species from the native pool. Similarly, the results observed for the two-species diversity treatment could not be attributed to a particular pair of species, but were instead due to 20 different random draws from the native species pool. Only the most diverse treatment (24 species) was consistently composed of the same set of species.

The effects of these diversity manipulations were remarkably clear: more diverse plots used and retained nutrients more efficiently than did less diverse plots (as measured by milligrams of nitrates per kilogram of soil in and below the rooting zone). The consequence of the reduced leaching and increased uptake of nitrate

It seems clear, then, that diversity does contribute to the sustainability and productivity of North American prairies. But the implications of this finding for policy regarding the protection of biodiversity will not become plain until more such studies are completed. For example, the 'benefits' of biodiversity were only observed in Tilman and colleagues' experiment as the number of species increased from one to ten species. Once diversity treatments exceeded ten species, there was no marked change in any measured aspect of community function. So do these results tell the policy maker to worry

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Biodiversity from Darwin on

WHILE applauding the experimental connection between biodiversity and grassland sustainability forged by Tilman and co-workers² (discussed in the main article here), it pays to recognize the long history of ideas about biodiversity. In the *Origin of Species*⁵, Charles Darwin wrote:

"It has been experimentally proved, if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and dry herbage can be raised in the latter than in the former case" (page 85).

It is not evident what experiments Darwin was referring to, but it is clear that he had a mechanism in mind for the connection between diversity and productivity. In particular, Darwin seems to have anticipated Tilman and colleagues' hypothesis of greater efficiency through niche diversification and more complete usage

of a limiting resource:

"... the truth of the principle that the greatest amount of life can be supported by great diversification of life, is seen under many natural circumstances" (page 85).

Experiments on biodiversity and ecosystem function also rest upon ideas advanced by some distinguished ecologists. For instance, the hypothesis that diversity begets stability was eloquently advanced by Charles Elton in 1958 (ref. 6). Elton thought that the balance of simple communities was more easily upset than that of richer communities — but he did not propose any clear mechanism for this effect. A turning point in diversity–stability research occurred with the publication of Robert May's theoretical monograph examining the dynamics of model ecosystems⁷. May irrefutably showed that, in itself, diversity provides no guarantee of stability, and that diversity

must be arranged in special ways to promote stable communities. Unfortunately, experimentalists seemed to misinterpret May's monograph as the final word on diversity–stability, and tended to abandon experiments aimed at contrasting the properties of diverse communities with those of simple communities.

It is only with the popularity of conservation biology and the awareness of worldwide threats to biodiversity that experimental ecologists are returning to the study of biodiversity's function. Further progress will require combining experimental expertise, as typified by the analyses carried out by Tilman's group, with Darwin's appreciation of natural history and the kind of theoretical insight exemplified by May's work. Simple experiments, by themselves, will not be sufficient to resolve the services and functions of biodiversity. It will pay to look to the past in tackling the relevant issues in the future.

P. K.

about preserving those first ten species of prairie plant, but not to bother once that quota has been satisfied? Obviously, there are other types of ecosystem function (resistance to drought or to pathogens, for instance) that could reveal a value to biodiversity in excess of the ten species seemingly needed for efficient nitrogen use.

Rather than viewing Tilman and colleagues' results as definitive documentation in favour of protecting biodiversity, we should see them as a call to develop more experiments of the same sort. We need to be especially conscientious about reporting experiments that fail to find any effects of biodiversity, and at finding ways to study systems such as forests that do not lend themselves to such elegant manipulative experiments. It is hard to be a biologist and not appreciate the diversity of life forms — but it is even harder rigorously to assess the ecosystem function of biodiversity in a manner that speaks plainly to the concerns of the public and policy makers. The new work is a milestone on the road to ecological research whose results can be directly related to debates about the preservation of Earth's variety of life forms.

There is currently great enthusiasm among biologists for using modern experimental approaches to identify potential values to biodiversity. But we need to keep this scientific evidence in perspective. No matter how the evidence weighs in with respect to the function of the variety of species in different ecosystems, there will remain many alternative reasons for preserving biodiversity. For example, some believe it is unethical to extinguish species forever, and several

religious leaders have supported the Endangered Species Act in the United States on moral grounds. Perhaps most important of all, many people value biodiversity as a legacy to be passed on to future generations — no one wants to tell their grandchildren that they passively watched as ignorance and greed led to the loss of richness of the world's flora and

fauna. The ecological value of biodiversity is of course a scientific issue — but it is not the only route towards delivering commitments to preserve the diversity of living things. □

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PARTICLE PHYSICS

A glimpse of the antiworld

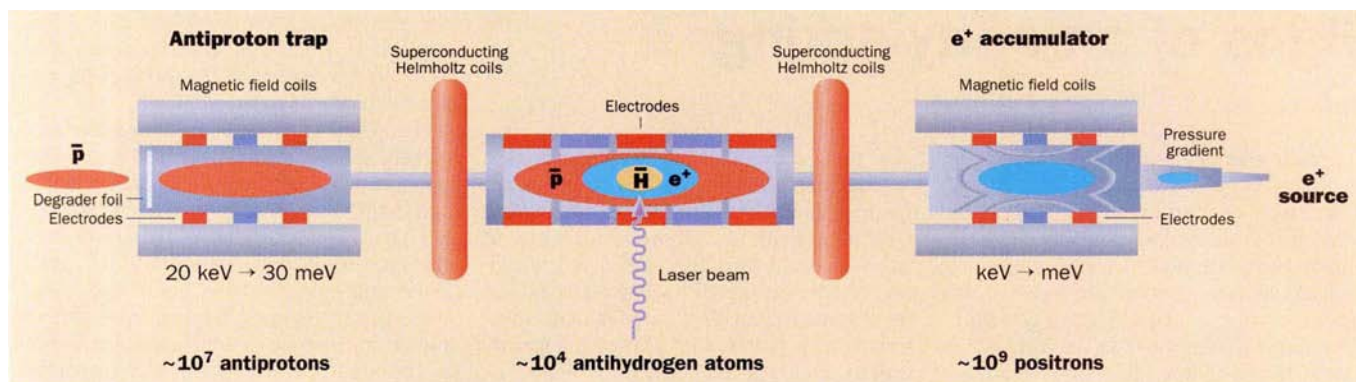
John Eades

THE *Physics Letters* article "Production of antihydrogen" by Baur *et al.*, published earlier this month¹, will not surprise any *Nature* reader who does not live at the bottom of a very deep mine. Indeed, the experiment made headlines from Bangkok to Toronto, and in most large villages in between. Why antihydrogen? Did, as *Le Figaro* implied, the CERN press service jump the gun in issuing a pre-publication announcement? Was there a European–American antihydrogen race? How significant is the experiment scientifically?

The electron's antiparticle, the positron (e^+), was found in 1932, but the antiproton ($\bar{p}$) only appeared on the scene in 1955. The possibility of binding these together to form an antihydrogen atom was immediately raised, in semi-popular articles^{2,3} as well as technical ones. However, there was no pressing reason to address the enormous problems of doing so until lasers revolutionized the practice

of atomic spectroscopy, making it possible to measure transition frequencies between atomic energy levels with almost unimaginable precision. At the 1992 Antihydrogen Workshop in Munich, it was suggested⁴ that the energy of the transition from the $1s$ to the $2s$ spectral state might ultimately be measured in ordinary hydrogen and antihydrogen to one part in 10^{18} .

Such comparisons test the world for its symmetry properties. A washing machine assembled from left-handed screws, coils and springs would work just as well as a right-handed one — together, the two form a left–right symmetrical system. However, the kit of parts for making a working, mirror-symmetrical model of the whole world is defective. Some essential screws and springs, like right-handed neutrinos, are missing. When physicists complained about this, nature pointed out that she had supplied a complementary kit of antiparticles (containing, for example,



A proposed CERN experiment to collect cooled antihydrogen in sufficient quantity to perform very precise spectroscopy. Bunched antiprotons with a momentum of $100 \text{ MeV } c^{-1}$ (energy 5 MeV) from a storage ring are collected in a Penning trap (an electromagnetic potential well) after being slowed to keV energies by passage through a metal foil. There, they are cooled to meV energies (corresponding to temperatures of a few hundred kelvin or less). Positrons from a radioactive source are also slowed

by collisions in a gas within which a pressure gradient is maintained, and collected and cooled in another Penning trap. The antiprotons and positrons are then emptied into a third trap where antihydrogen atoms are formed. Inhomogeneous magnetic fields superimposed on this trap create a force on the positron magnetic moment that always pulls the anti-atoms towards the central region, eliminating wall collisions. The almost stationary atoms can then be probed by laser beams.

right-handed antineutrinos) for just this purpose, and that they were using the wrong mirror. The correct one should replace particles by antiparticles and reverse velocities, as well as turn left into right. These operations are abbreviated by C, T and P, for charge, time and parity, and physicists are still testing this overall CPT symmetry (the well-known but still mysterious CP-violation in the $K^0-\bar{K}^0$ particle-antiparticle system is compensated by a simultaneous T-violation, leaving the joint CPT symmetry untouched).

One part in 10^{18} is not really an outrageous target when crucially important effects at the 10^{-15} level (such as the mass difference between long and short-lived kaons, and the hyperfine splitting of the hydrogen atom ground state) are already known. Physicists therefore tend to regard the symmetry already established at the one-in- 10^{18} level in the $K^0-\bar{K}^0$ particle-antiparticle system as a yardstick for CPT checks in other systems, rather than as an argument against making them.

Then there is gravity. Gravitational fields change the dielectric constant of spacetime by squeezing, twisting and otherwise distorting it. They also modify the energy-momentum tensor of matter. For an atom in a gravitational field, the net result is a redshift in its spectral frequencies. However, CPT symmetry does not require that this be the same for hydrogen and antihydrogen, only that the hydrogen shift produced by an object's field be equal to the antihydrogen shift produced by the corresponding anti-object's field. So antihydrogen gravitational anomalies in a matter field (such as that of the Earth or Sun) and CPT violations would both result in a $1s-2s$ transition frequency for antihydrogen that differs from the hydrogen one.

Gravity rather than CPT would be incupulated if an annual modulation of such a difference occurred, as the Earth's elliptical orbit moved the atoms up

and down in the Sun's gravitational potential⁵. In this case, the two frequencies would have been redshifted in the local gravitational potential by different amounts, thereby violating the equivalence principle of general relativity. A one part in 10^{18} frequency difference would then be ascribable to a one in 10^9 equivalence violation.

The nine antihydrogen events reported by spokesman Walter Oelert and his colleagues¹ are the fruit of a suggestion made at the Munich workshop⁶ that high-energy antiprotons passing through atoms could occasionally convert some of their energy into electron-positron pairs. In rare cases, they would hang on to the positron and emerge as an antihydrogen atom with almost the same energy they started with. The cross-section for this is minuscule ($\sim 10^{-33} \text{ cm}^2$), but the rate could be increased by squirting the target atoms across the path of antiprotons circulating in a storage ring. Moving at almost light speed, these would pass through the target several million times per second. A Fermilab experiment was expected⁶ to produce 600 antihydrogen atoms in 1995 with a hydrogen jet, but delays in this project left a clear field for the experiment of Oelert and colleagues at CERN.

In the experiment, antiprotons in LEAR, the Low Energy Antiproton Ring, traversed a xenon jet and created antihydrogen atoms, as described above. Being neutral, these atoms left the storage ring instead of being magnetically steered around the next bend. After a 12-m flight path, the atoms were disassembled and each e^+ stopped in a silicon detector while the $\bar{p}$ continued into a magnetic spectrometer. Cross-correlation between the gamma-ray energies from the e^+ annihilation and the $\bar{p}$ momentum and flight time over a second flight path determined unambiguously that nine events had occurred in which both particles came

from an antihydrogen atom.

A price has been paid for this relatively easy ride. Antihydrogen atoms near light-speed will leave any apparatus of manageable size in a few tens of nanoseconds, whereas high-precision $1s-2s$ spectroscopy requires them to stand still in a laser beam for much longer than the lifetime of the $2s$ excited state (about 0.14 of a second). The present experiment has therefore only lifted the veil hiding the antiworld, and it will be left to Fermilab to do what spectroscopic measurements can be made with nanosecond-lifetime antihydrogen atoms (a measurement of the Lamb shift for example) after LEAR closes in December. Ultra-high precision spectroscopy will have to wait until antiprotons and positrons can be slowed to milli- or micro-electron volt energies, and the resulting antihydrogen atoms levitated to eliminate collisions with the walls of the vessel they are created in. Such plans are now being laid at CERN (see figure) although they will require an *ad hoc* replacement for LEAR.

And the ethical issues? CERN successfully resisted media pressures to announce the result before the *Physics Letters* refereeing procedure was complete. Meanwhile, Oelert patiently answered the referee's questions by electronic mail shortly before Christmas from a conference in Tokyo, but otherwise said nothing. Full marks all round for ethics. □

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Signs of an early spring

Ian D. Hutcheon

THE most chemically primitive objects in the Solar System are widely recognized to be type 1 carbonaceous chondrite meteorites (commonly known as CI chondrites), whose abundances of non-gaseous elements closely approximate those of the Sun. Much of our knowledge of the average chemical composition of the Solar System is based on their investigation¹. Despite the fact that these meteorites have retained their original chemical composition and contain well-preserved mineral grains of presolar origin², the CI chondrites are not pristine samples of material from the solar nebula. Instead, they show abundant evidence of chemical processing^{3,4} in an aqueous environment on the asteroid-size 'planetesimals' where these meteorites aggregated. This aqueous activity was generally thought to have occurred within ~100 million years (Myr) of the time of formation of the first meteorites⁵ but the detailed timing has

not, until now, been tightly constrained. As they describe on page 701 of this issue, Endress *et al.*⁶ have now dated the formation of individual carbonate minerals in two CI chondrites, and shown that aqueous activity on the CI parent planetesimal must have begun very early in Solar System history, within ~20 Myr of the formation of the first solid bodies.

A central goal of meteoritics is understanding the chronology of events leading to the transformation of the original interstellar material inherited by the solar nebula, which formed meteorites and then small planetary bodies by the processes of evaporation and condensation, melting and recrystallization, accretion, brecciation (the incorporation of small rocks into a matrix) and metamorphism. The timescale of these early Solar System events (~1–10 Myr) is below the resolution of the conventional radiometric dating systems used on the Earth — events in

the early Solar System separated by 1 Myr require a fractional time resolution of $\sim 2 \times 10^{-4}$, equivalent to about 3 days at the birth of a 45-year-old.

Establishing a time sequence for such closely spaced events requires a different approach, using short-lived radionuclides such as ^{26}Al ($t_{1/2} = 0.73$ Myr) or ^{53}Mn ($t_{1/2} = 3.7$ Myr). These species potentially provide the time resolution needed to date events during the first 10 Myr of Solar System history. Both ^{26}Al and ^{53}Mn are now extinct, but their presence in the early Solar System is revealed⁷ by excesses of the respective daughter isotopes, ^{26}Mg and ^{53}Cr , in the very oldest meteoritic materials.

In the study by Endress *et al.*, chromium isotope compositions of individual carbonate fragments from the Ivuna and Orgueil CI chondrites were measured using secondary-ion mass spectrometry, a technique in which a finely focused beam of energetic ions sputters microscopic amounts of material from an area only a few micrometres in diameter to determine chemical and isotopic compositions. Carbonates selected for this study are manganese-rich and chromium-poor, and appear to have been deposited in veins in the rock of their parent bodies during the early stages of aqueous alteration^{3,5}. Aqueous alteration occurs when salt-rich fluids analogous to terrestrial brines, produced by the partial dissolution of existing minerals by liquid water, circulate through the CI parent body causing chemical redistribution and the growth of new minerals.

As the authors discuss, the carbonates contain very large excesses of ^{53}Cr from the decay of ^{53}Mn , with $^{53}\text{Cr}/^{52}\text{Cr}$ ratios as high as twice the normal value. From the linear correlation between the ^{53}Cr excess and the $^{55}\text{Mn}/^{52}\text{Cr}$ ratio, Endress *et al.* infer the initial abundance of ^{53}Mn , and find a $^{53}\text{Mn}/^{55}\text{Mn}$ ratio of $\sim 2 \times 10^{-6}$ at the time of carbonate formation. By comparing this with ratios measured in other meteoritic materials, they conclude that CI carbonates formed only 16–18 Myr after the calcium- and aluminium-rich inclusions (CAI) believed to be the first solid objects formed in the Solar System⁸. This interval is substantially shorter than previously believed⁵ and has important implications for the accretion and subsequent thermal history of the CI parent planets.

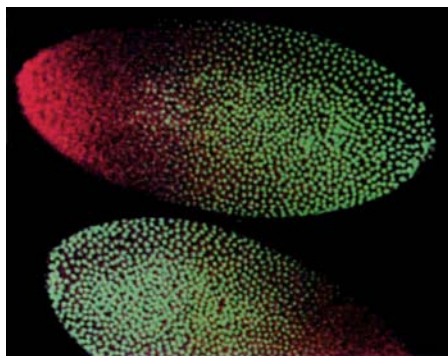
Aqueous activity requires temperatures high enough to melt ice, so by shortening the timescale by up to a factor of five, the new observations require rapid accretion and thermal evolution of the typical CI parent body, from initial temperatures below 150 K to temperatures of about 420 K (ref. 9) on a timescale no longer than 10–15 Myr. Impacts are unlikely to have provided enough heat over this period, so aqueous alteration of the CI chondrites was probably driven by heat released from ^{26}Al decay⁴.

These new observations of radiogenic

GENE EXPRESSION

RNA bound to silence

AFTER fertilization, the *Drosophila* embryo does not begin cell division as do most other embryos. The nucleus of the fertilized egg divides rapidly — without actual cell division — and a syncytial blastoderm is formed from the resulting monolayer of nuclei. The basic instructions for the polarity of the embryo are found in the oocyte itself, with the maternal messenger RNA for bicoid, attached to the egg's anterior tip, determining the position of the future head of the fly. On pages 694 and 746, Dubnau and Struhl and Rivera-Pomar *et al.* show that bicoid does more than we thought.



Bicoid and caudal are homeodomain proteins that bind DNA sequences and regulate transcription of specific target genes. After the bicoid maternal mRNA is translated, the bicoid protein gradient (stained red in the early embryos pictured here) forms, spreading out in an anterior-to-posterior fashion. Shortly after, the caudal posterior-to-anterior protein gradient (stained green) forms. Caudal mRNA, like that of bicoid, is provided by the mother and stored in the egg until it is needed for embryonic development. Unlike bicoid, however, caudal mRNA is evenly distributed throughout the egg. So how does the caudal protein gradient — without which normal patterning is compromised — form?

Together, the two papers in this issue show that bicoid is necessary for formation of the caudal gradient, and why. It turns out that bicoid's DNA-binding domain can also bind RNA, regulating gene expression at a level never before seen for a homeodomain — translation. The homeodomain of bicoid binds to regulatory sequences in the 3' untranslated region of caudal's mRNA. Binding these sequences blocks translation initiation, preventing production of caudal protein. Thus the complementary bicoid and caudal gradients are formed, with less caudal being translated wherever there's more bicoid.

Homeodomain proteins, first described in *Drosophila*, are involved in establishing the basic body plans of all animals (and some plants), but up to now they have always been seen as transcription factors binding DNA. Future work may show that bicoid is not the only RNA-binding homeoprotein.

Kimberly Carr

^{53}Cr allow the timescale of carbonate formation and aqueous activity on the CI parent body to be set in the context of other early Solar System events. The initial $^{53}\text{Mn}/^{55}\text{Mn}$ ratio inferred for the CI carbonates^{6,10} is very similar to the values reported for four classes of differentiated meteorites^{8,11–13} (which come from larger parent bodies, where temperatures rose enough for the rocks to melt and separate under the force of gravity), but it is distinctly lower than the values found for CAI in the Allende carbonaceous chondrite⁸ and in several other chondrites¹⁴. These differences imply that the chondrites are 4–7 Myr younger, and the CI carbonates and differentiated meteorites 14–18 Myr younger, than Allende CAI — consistent with the interpretation of CAI as representative of the first Solar System solids.

The ~10 Myr difference in age between chondrites and several classes of differentiated meteorites may reflect a fundamental change in early Solar System activity, from nebular processes such as condensation and chondrule formation (precursors of chondrite formation) to igneous differentiation and thermal metamorphism occurring in a planetary environment. The observation of high levels of ^{53}Mn in CI carbonates indicates that aqueous alteration occurred at a very early stage, perhaps at the same time as parent body accretion. This shortened timescale fits well with current estimates of the initial abundance of ^{26}Al (ref. 7) and with thermal evolution models for ordinary chondrites.

While a complete picture is still beyond our grasp, many lines of evidence now suggest rapid growth of solid bodies, from centimetre to kilometre sizes, very early in Solar System history and within ~20 Myr of the formation of the first solids. □

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Psychoactive smoke

Alexander H. Glassman and George F. Koob

It is known that levels of the enzyme monoamine oxidase (MAO) are reduced in the platelets of cigarette smokers and that they return to normal when smokers give up the habit. Monoamine oxidase is involved in the degradation of the biologically active monoamines (neurotransmitters such as dopamine, noradrenalin, serotonin and phenethylamine) and exists in two isozyme forms, A and B.



Punch/Mary Evans

Smoking gun: among other issues, the study of Fowler et al.¹ raises the question of whether cigarettes are a 'gateway' drug leading to addiction to other drugs of abuse.

On page 733 of this issue¹, Fowler and her co-workers extend the connection between MAO and smoking, and bring it from the platelet into the brain. They have used *in vivo* imaging techniques to show that, compared to non-smokers, the brains of people who currently smoke show a 40 per cent reduction in the B form of the enzyme; moreover, levels of MAO B return to normal in former smokers. MAO B is selective for the metabolic degradation of dopamine so that its inhibition would increase the functional availability of this neurotransmitter. The findings are of interest in several respects that bear not only on general issues to do with addiction, but also on the association between cigarette smoking and a reduced risk of developing Parkinson's disease².

Although not all studies find a reduced rate of Parkinson's disease among smokers³, most do; on average, it seems that smokers have only half the risk of contracting the disease compared with non-smokers. Parkinsonism is characterized by a decrease in dopamine function and loss of dopamine neurons. L-deprenyl

(selegiline) is an inhibitor of MAO B that reduces the symptoms and may slow the course of the disease^{4,5}, presumably by affecting the functional levels of dopamine. Only one of the eight smokers in the study shows a degree of MAO B inhibition similar to that produced by therapeutic doses of L-deprenyl. Even if this smoke-induced reduction is responsible for the lower rate of Parkinsonism among smokers (the neuroprotective effect), the mechanism is still not clear. It could be that smoking-induced inhibition of MAO B reduces peroxidase formation and the associated oxidative damage at levels of inhibition less than those required for the therapeutic effects of L-deprenyl in symptomatic Parkinson's patients.

The authors also raise the question of whether the reduction in MAO B activity in the brain is involved in increasing the addictive properties of cigarettes. Dopamine plays a key role in reward signalling and addiction⁶. The mesolimbic dopamine system that runs from the ventral tegmental area to the basal forebrain has been implicated in the rewarding properties of almost all drugs of abuse, including cocaine, amphetamine, heroin, alcohol and nicotine. If dopamine availability is significantly altered by MAO B inhibition, this could influence the vulnerability of an individual or animal to various aspects of addiction.

Addiction can be defined as compulsive use of a drug resulting in loss of control over intake⁷, and can be studied in animals by measuring their tendency to self-administer a drug when given the opportunity to do so. L-deprenyl alone does not seem to have addiction potential in this assay, nor does it increase the reinforcement potency of cocaine or methamphetamine^{8,9}. Also, clinically relevant doses of L-deprenyl do not substitute for the subjective stimulus effects of cocaine, amphetamine or methamphetamine in animals as measured by a procedure where animals choose a specific response based on their drug state^{9,10} (known in behavioural pharmacology as drug discrimination). At first sight, this might suggest that inhibition of MAO B is not a major determinant of addiction. However, the self-administration studies looked only at the effect of acute injections of L-deprenyl, whereas the effects of chronic L-deprenyl treatment on drug self-administration are unknown. It therefore remains possible that chronic lowering of MAO B, and any resulting increased availability of synaptic dopamine, could facilitate nicotine self-administration or addiction.

Animals clearly self-administer intravenous nicotine, free of any smoke vehi-

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cle, and MAO B inhibition is not crucial in addicting animals to nicotine¹¹. However, individual animals of any given strain vary in their responses to addictive drugs¹², and their vulnerability to addiction is strongly influenced both by environmental effects, such as stress, and by personality factors such as a tendency, or lack of it, to novelty seeking (exploratory, thrill-seeking and excitable behaviour)¹³. Curiously, even before they manifest the disease, people who develop Parkinsonism show less novelty-seeking behaviour than do control populations¹⁴. Although novelty seeking and addictability have been linked in animals, there is only limited evidence that this link is mediated by dopamine¹⁵⁻¹⁷. Nevertheless, it is seductive to speculate that the biological variability in dopamine, that makes animals that are less prone to seek novelty less prone to addiction, is the same that makes the Parkinson's patient less likely to smoke.

The observations of Fowler *et al.* raise the additional issue of whether this ability of cigarette smoking to inhibit MAO B and augment dopamine release might be the basis of the strong association seen between cigarette smoking and alcoholism. The epidemiological data consistently show that alcoholism in particular, and use of other drugs of abuse in general, are extremely likely to be linked with smoking. In animals, previous exposure to stimulant drugs, including nicotine, has been shown to reduce the latency to the acquisition of self-administration¹⁸. If, in

fact, cigarette smoking is able to further augment dopamine release through MAO B inhibition, and this contributes to increasing an individual's likelihood of addiction to other drugs of abuse, it would give a neuropharmacological basis to the proposal that cigarettes are a 'gateway' drug. If this is true, it would raise even further the concern about adolescent exposure to cigarettes.

Cigarette smoking has also been long associated with increased alertness, sustained performance in situations of fatigue and increased cognitive performance. Although these characteristics are clearly confounded in individuals dependent on smoking by the decreases in performance stemming from nicotine withdrawal, there is increasing evidence that nicotine itself can have significant cognitive-enhancing properties in the absence of nicotine dependence¹⁹⁻²¹. The demonstrated cognitive-enhancing properties of L-deprenyl in human subjects²² suggest that another contribution to the enhancement of cognitive performance by smoking may be the reported decrease in

MAO B activity. This hypothesis could also be tested experimentally.

In all, the observations of Fowler *et al.* open a new vista on the biological effects of cigarette smoking. Chronic MAO B inhibition in the brain may explain the resistance of cigarette smokers to Parkinson's disease, and may contribute to the performance-enhancing properties of cigarette smoking. It is also conceivable that MAO B inhibition is involved in the development of cigarette addiction and in a propensity to co-addictions. The active ingredient in the smoke responsible for these actions is unknown, but it is becoming increasingly evident that nicotine is not the only psychoactive component of tobacco smoke. □

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GALAXY FORMATION

A distant but absorbing youth

Antoinette Songaila Cowie

It is some measure of the current level of excitement in high-redshift galaxy studies that a single object could have been the subject of two press releases and some five papers in the past three months. The fascinating subject of all this activity was discovered as a Lyman-alpha absorption line (betraying the presence of hydrogen) at a redshift of 4.4 in the spectrum of the quasar BRI1202-0725 (refs 1 and 2). Damped Lyman-alpha systems (DLASs) are produced when a gas cloud with a hydrogen atom column density of about 10^{21} cm^{-2} lies in front of the quasar — not an uncommon event at lower redshift, but the system in front of 1202-07 is the highest-redshift example known by far, from a time when the Universe was about a tenth of its present age. The excitement stems from the possibility that this may be a galaxy in an extremely early stage of formation, perhaps only a few tens of millions of years old. But is it really so?

An earlier press release³ described low-resolution spectroscopy and high-resolution imaging by D'Odorico and collaborators and cited two supportive pieces of evidence: the presence of trace levels of heavy elements, a certain sign of star formation, in the damped Lyman-alpha system itself; and the discovery, extremely close to our line of sight to the quasar, of a small object whose colours put its redshift (z) between 4.0 and 4.7, making it a prime candidate for identification with the early

galaxy. But since then part of the picture has weakened. Spectroscopy^{4,5} and photometry⁶ of the continuum object suggests very strongly that it is at the quasar's redshift of $z = 4.7$ (where some of its ionization could be produced by the quasar, and it may be illuminated, in part, by scattering of the quasar's light) and so out of contention as the source of the Lyman-alpha absorption.

Although some other object in the field might be the missing galaxy, Limin Lu and collaborators⁷ at Caltech are left the task of making the case for an early galaxy based only on the abundance and kinematics of the heavy elements detected in the absorption line system. The gist of their argument is that the proportion of 'metals' (by which astronomers mean elements heavier than helium) is about what would be expected of a galaxy where only the most massive stars, greater than about 10 solar masses, have reached the end of their lives, and by exploding as supernovae or through strong stellar winds have enriched the gas in the galaxy with the heavy elements synthesized within them.

The case is still quite strong. Spectacular high-resolution spectra, taken with the HIRES spectrograph at the Keck 10-m telescope in Hawaii, show absorption in common metal lines from a system in many ways remarkably like its counterparts⁸ near $z = 2$. Current understanding is that these low-redshift DLASs may be

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the early stages of disk galaxy formation. Art Wolfe and his collaborators have strongly argued this case, based on probable sizes, masses and kinematics, and it is supported by the rough equivalence of the total amount of baryonic material in the DLASs at $z = 2$ and in present-day disk galaxies. Indeed, a beautiful study has demonstrated⁹ that the amount of baryonic material (ordinary matter made mostly of protons and neutrons) in DLASs remains constant from $z > 4$ down to $z = 2$, before making a precipitate drop to the current epoch, presumably as a result of the conversion of gas to stars in these disks. Two recent identifications¹⁰ of spiral galaxy counterparts to DLASs at $z < 1$ strengthen the picture.

But this apparent conservation of material causes intriguing problems for the interpretation by Lu *et al.* of the 1202–07 system as a massive spiral galaxy at the beginning of its star formation, and with the history of the DLASs in general. The most obvious is that if DLASs start to form metals so early, why do we not see cases at smaller redshifts with higher metal enrichment? The answer might be that the dust that accompanies the earliest generations of disk stars obscures the quasars behind these systems from further view, so that only the newly-formed DLASs are visible¹¹, but it might also be that these are not star-forming objects. Another interesting issue, which Lu *et al.* raise, concerns the formation of the 1202–07 system: if the line structure really indicates rotation in the disk of a galaxy as massive as 10^{11} solar masses, then important constraints will be placed on the currently favoured mixed dark matter scenario of structure formation, which predicts that few objects this massive should be present so early. However, the kinematic structure could have an origin other than gravitational motion, and we should probably wait to see whether other DLASs at these redshifts behave similarly before coming to a final conclusion.

Of course, the strongest case for this object's being a galaxy is the presence of metal lines: metals must mean stars and stars must mean galaxies — or must they?

Nearly all intergalactic-medium clouds we can study¹² at redshifts greater than 2, not just the DLASs, have metal enrichment at about the level seen by Lu and colleagues. Is there ongoing star formation in all of these objects, or is this the sign of uniform pre-galactic enrichment by a first generation of stars in the intergalactic medium — the so-called population III? Direct confirmation of ongoing star formation can in the end come only from detection of a continuum object, or line emission

from a DLAS, something that has been ferociously difficult to obtain at high redshift. Perhaps now with the new 10-m telescopes such as the Keck we will begin to make these identifications, and push our knowledge of galaxy formation to these extreme redshifts. □

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OCEANOGRAPHY

Deep water distillation

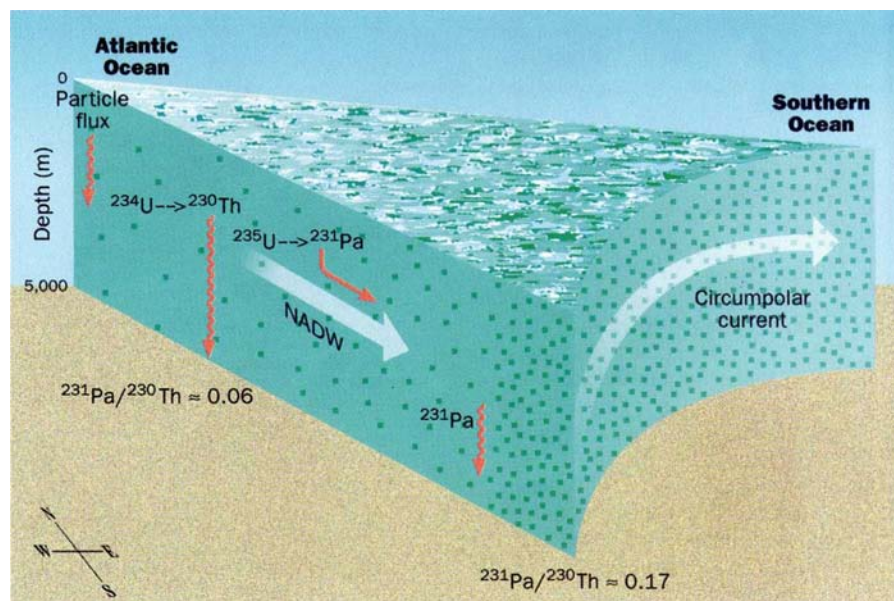
Ed Boyle

By effectively distilling a particular pair of radioisotopes deep under the Atlantic, ocean circulation has recorded its own strength thousands of years into the past. On page 689 of this issue¹, Yu *et al.* report the discovery of this process and what it tells us about the state of the oceans during the last ice age.

The North Atlantic Deep Water (NADW) is an ocean current that leaves the surface of the northernmost Atlantic and is carried into the furthest reaches of the deep global ocean. It has considerable consequences for the climate. The heat it releases to the polar Atlantic warms Europe, and it serves as a source of heat and salt to the Antarctic Southern Ocean. It has sometimes been argued that this current stopped flowing during times of glaciation; others have made the case that it continued at slightly reduced (or per-

haps even equal) intensity. Yu *et al.* add a surprising new twist to the debate based on ratios of protactinium-231 to thorium-230 in oceanic sediments. They conclude that there must have been some form of the NADW conveyor operating during the Last Glacial Maximum, and transporting about as much water as the current does now.

Originally, the sedimentary $^{231}\text{Pa}/^{230}\text{Th}$ ratio was used for geochronology². Both of these isotopes are generated at precisely known rates in sea water by their parent uranium isotopes ^{235}U and ^{234}U , whose oceanic distribution is uniform and well known. Once formed they attach themselves relatively quickly to particles (mainly particles of biological debris) and sink to the sea floor, until they disappear by radioactive decay (with half-lives of 32,500 and 75,000 years, respectively).



The flow of the North Atlantic Deep Water (NADW) from the Arctic to the Antarctic. Uranium isotopes in the water decay to produce ^{231}Pa and ^{230}Th , and in the course of the journey most of the ^{230}Th attaches itself to sinking particles and is deposited in Atlantic sediments. Being less reactive, ^{231}Pa travels further, and much of the Atlantic ^{231}Pa is captured by the high particle fluxes of the Southern Ocean. The ratio of these isotopes in old sediments shows that, contrary to some theories, there was an Atlantic current of a similar scale during recent ice ages.

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The chronological method assumed that the precisely known generation rates should lead to a constant $^{231}\text{Pa}/^{230}\text{Th}$ ratio in young oceanic sediments, and that the ratio should decay exponentially with age in older sediments. Knowing the initial surface sediment ratio and the ratio in deeper sediments, the age of each deep sediment sample could be calculated.

This use of sedimentary $^{231}\text{Pa}/^{230}\text{Th}$ seemed unassailable until other dating methods showed that it assigned incorrect ages³⁻⁵. Further investigations showed that surface sediments do not start with constant $^{231}\text{Pa}/^{230}\text{Th}$: the ratio shows large variations from place to place and at the same site over time⁶⁻⁸. This variability was a puzzle whose cause has only become evident in recent years with studies of the oceanic chemistry of these isotopes⁹⁻¹¹. Although both isotopes are 'particle-reactive' and attach themselves to particles which sink to the bottom, it has become clear that ^{230}Th is considerably more particle-reactive than ^{231}Pa . At particle flux levels typically seen in large ocean basins, ^{230}Th attaches itself to particles and is removed from the water column in a few decades; ^{231}Pa is removed on a timescale of a century or more.

As a result of this difference in reactivity, most ^{230}Th falls to the bottom almost immediately below the site where it is generated, but a significant fraction of the ^{231}Pa is transported laterally to sites of higher particle flux. For this reason, in recent years it has been considered that sedimentary $^{231}\text{Pa}/^{230}\text{Th}$ is an indicator of past biological productivity¹²⁻¹⁴ because it is strongly correlated with total particle flux.

In a study that was initially aimed at using this indicator to examine changes in Atlantic biological productivity patterns between the present and the Last Glacial Maximum, Yu *et al.* uncovered a surprising result: there is ^{231}Pa missing from the Atlantic Ocean. Almost all of this isotope must reside in oceanic sediments, because its concentration in sea water is lower than would be implied by its production rate. But Yu *et al.* found ^{231}Pa to be clearly deficient throughout the surface sediments of the Atlantic Ocean, with an average $^{231}\text{Pa}/^{230}\text{Th}$ more than 30 per cent below the production ratio. Conversely, they noted that $^{231}\text{Pa}/^{230}\text{Th}$ was clearly increased in the surface sediments of the Southern Ocean by about the same amount. They surmise that the ^{231}Pa missing from the Atlantic Ocean is conveyed into the Southern Ocean by NADW convection, to be removed there by the veil of particles created at the Antarctic Circumpolar Front.

This surprising deduction is compounded by their further discovery that the same pattern of Atlantic depletion and Antarctic enrichment is seen during the Last Glacial Maximum. They con-

clude that the NADW (or more likely a shallower analogue) continued to export Atlantic waters into the southern and global oceans. This qualitative result is buttressed by their quantitative analysis which examines the sensitivity of ^{231}Pa export from the Atlantic to changes in deep-water flow rates and changing productivity. The behaviour of ^{231}Pa makes sense in terms of the time NADW spends in the Atlantic (about two centuries). Decade-scale resident ^{230}Th cannot travel far along the conveyor, but century-scale resident ^{231}Pa can move out of the basin in the time available. The export rate of the NADW-analogue during the Last Glacial Maximum must have been close to the present rate.

A persistent sceptic could argue that ^{231}Pa is not actually missing from the Atlantic (either at present or during glacial times), and that the apparent deficiency is an artefact of the limited number of sites analysed. Yu and colleagues argue persuasively that this alternative is not probable, and so present a clear challenge to the sceptics: go out and find the missing ^{231}Pa .

Many investigators suspect that the NADW current is a key control on natural climate change during ice ages. If the results of Yu *et al.* are accepted, then that control must be exerted through changes in the mode of circulation (shallower or deeper flow, for example), rather than by an on-off cycle or any influence on global deep water characteristics.

Some insight could be derived from more complicated models with more degrees of freedom. Further data and modelling will either confirm and strengthen the results of Yu *et al.* or improve our understanding of oceanic radionuclide behaviour — a win-win situation. □

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Romantic airs

THOSE who have made love in an aircraft speak highly of the experience; indeed, they claim membership of the exclusive 'mile-high club'. Daedalus connects this fact with the bizarre rituals of certain dedicated masturbators, who half-suffocate themselves with ropes or plastic bags to enhance the sensations of orgasm. Sometimes they overdo it, and are later found tragically dead. Both suffocation and aircraft altitudes, of course, reduce the level of oxygen in the body. It seems that sex is sexier with less oxygen.

Daedalus has an explanation. The biomolecule nitric oxide is a key mediator in penile erection and other aspects of the sexual process. It is destroyed by oxygen, which takes it to dinitrogen tetroxide (or in the body, to nitrate). Removing oxygen must extend the biochemical lifetime of nitric oxide, and intensify the sexual experience.

Artificial sources of nitric oxide seem to have the same effect. Nitrite stimulants, and even nitroglycerine anti-angina tablets, have been recommended as sexual aids. Yet such powerful sources of nitric oxide can have worrying side effects. It would seem better to extend the lifetime of the body's own nitric oxide, if a less drastic way can be found than oxygen starvation.

Daedalus notes that carbon monoxide reverses the destruction of nitric oxide by oxygen. It is preferentially oxidized instead, and should greatly extend the lifetime of nitric oxide in the body. A mere trace of carbon monoxide, comparable to the levels of nitric oxide in the body itself, should do — even the amount inhaled with cigarette smoke might be adequate. Indeed, this may explain the deplorably sexy image of the cigarette, and the power of the car as a vehicle of courtship (its exhaust contains both oxides). If so, the catalytic exhaust-purifier signals the end of a dream.

Daedalus is now testing these notions. He is trying out various mixtures of oxygen-depleted air, nitric oxide and carbon monoxide on volunteers, seeking the formulation of greatest sexual impact. With luck oxygen depletion will turn out to be unnecessary, while the other gases will have their maximum effect at fairly harmless levels.

DREADCO will then market an inhaler which leaks these gases into the user's airstream. It will not obstruct normal breathing; the user will run no risk of suffocation. Daedalus is planning a neat design modelled on a classic sophisticated cigarette holder. The elegant seductions of old movies will be replayed for real in thousands of bedrooms.

David Jones

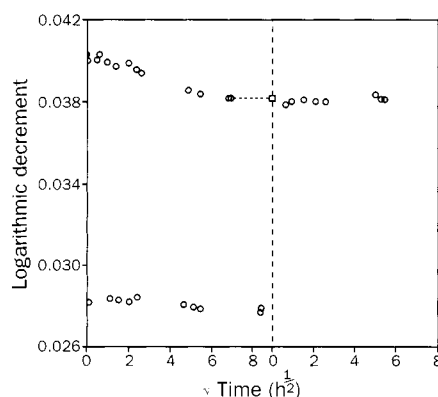
Why old fiddles sound sweeter

SIR — Musicians often claim that regular playing of a stringed instrument improves its tone¹. Although sound quality is subjective, it is often thought desirable to have high stiffness-to-weight ratio and low damping coefficient²⁻⁴, as are found in the mature pine and spruce commonly used for sounding membranes. Here we show that for spruce, at higher humidities, continuous forced vibrations cause the stiffness to increase and the damping coefficient to decrease, thus lending support to the musicians' claim. The changes are ascribed to slow redistribution of moisture to lower-energy sites.

We subjected beams of spruce ($85 \times 12 \times 0.5$ mm) to continuous vibrations at the natural frequency of 10 Hz (see figure). The damping coefficient was found to drift downwards by about 5% on average, sometimes after an initial small increase. When the forced vibrations ceased, there was little change. The forced vibrations always caused an increase in the natural frequency of about 0.3%. At 44% relative humidity (RH), the forced vibrations showed little effect considering the precision of the experiment.

The damping coefficient of wood is higher at higher humidities, with typically a 3.5% increase in damping coefficient for each 1% rise in wood moisture content. Measurements during continuous vibrations with simultaneous step-humidification from 80% to 90% RH showed a very rapid increase in damping coefficient followed by a slight drift downwards. The increase was more rapid with forced vibrations than without, typical time constants for the same test piece being approximately halved by the forced vibrations (for example, 0.4–0.15 h). The time constant for the moisture content increase of a matched weighed sample was typically 3 h.

The explanations for the various anomalies observed may lie with the mechanisms of moisture bonding. On the basis of sorption isotherms, Kollmann⁵ suggested that sorbed water could be divided into three groups: monolayer adsorption at low humidities, multilayer adsorption starting at intermediate humidities, and relatively free capillary water, which predominates at higher humidities below the fibre-saturation point. The rapid increase in damping during humidification may result from the breaking of bonds by internal swelling strains. The effect of



Damping coefficient expressed as logarithmic decrement plotted against the square root of time during forced vibrations at the natural frequency of a spruce cantilever beam of dimensions 85 mm (axial) by 12 mm (radial) by 0.5 mm (tangential), at 90% RH (upper) and 44% RH (lower). The vibrations were switched off after 48 h. Left, with vibrations; right, vibrations stopped. Measurements were made following moisture equilibration (to an estimated tolerance of 0.3%). The time at equilibrium humidity was at least 16 times the measured sorption time constant. At 90% RH, the experiment was continued by switching off the vibrations after 48 h. The coefficient of variation of the measurement technique was 0.35% for the damping coefficient and 0.0065% for the frequency. The humidity of the environmental chamber was maintained within $\pm 1\%$ RH and $\pm 0.1^\circ\text{C}$. The measurements made during continuous forced vibrations at constant humidity were made six times, on matched samples.

the simultaneous forced vibrations may be to raise the energy levels at many of the bonding sites and so accelerate the bond-breaking process that must accompany the moisture-induced swelling.

The slow decrease in damping and increase in stiffness during vibrations at constant high humidity may be the result of slow re-location of water molecules from high-strain, and therefore high-energy, sites, to lower-energy sites. The fact that little change takes place at 44% RH suggests that it is mainly the multilayer water molecules that are re-located. It was found that the changes could be reversed by drying the samples at 30% RH and then re-humidifying.

Although, for the best care of a musical instrument, both high and low humidities should be avoided, the above results suggest that at intermediate or high humidities the sound quality may be improved by regular playing.

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Similar bacteria in remote oil fields

SIR — L'Haridon *et al.* recently reported the isolation of thermophilic microorganisms from oil wells¹. Besides 'marine' hyperthermophilic *Archaea*, they have isolated several bacterial strains that can be ascribed to the *Thermotoga*, *Thermoanaerobacter* or *Thermodesulfobacterium* genera. This bacterial flora is very similar to that isolated from another oil well, located in the Paris Basin^{2,3}. That well had similar depth, pressure, temperature and water salinity characteristics.

Very closely related bacteria were also isolated from oil wells located thousands of kilometres apart. *Thermotoga elfii*⁴, isolated from an oil well in the Cameroons, and *Thermotoga subterranea*, described by L'Haridon *et al.*¹ and Jeanthon *et al.*⁵, may well represent the same species. They display the same morphological, physiological and metabolic characteristics, and share 98.2% of their 16S ribosomal RNA sequence. *T. elfii* with similar characteristics has also been isolated from several other oil fields (my unpublished data). These geographically remote oil fields constitute geologically isolated and physicochemically peculiar ecosystems. Considering the close physiological adaptation of the bacteria to their habitat², they are unlikely to have been accidentally introduced into the oil fields during recent drilling operations, with well equipment or in subsequent production operations. All these observations strengthen the hypothesis that these microorganisms are indigenous^{1,2}.

Several important questions remain. For example, where are these bacteria from? How did they succeed in colonizing these habitats? Note that *Thermotoga*, as well as other mesophilic anaerobes we have isolated from various oil fields (manuscript in preparation), represent the most ancestral branches of the bacteria. Are these microorganisms directly descended from bacteria that were trapped during the formation of the oil, or accompanied its migration through tens to hundreds of millions of years? Did they arrive in the oil field later as a consequence of aquifer activity? What is their mode of maintenance and development in their environment? Much work is needed to answer these questions.

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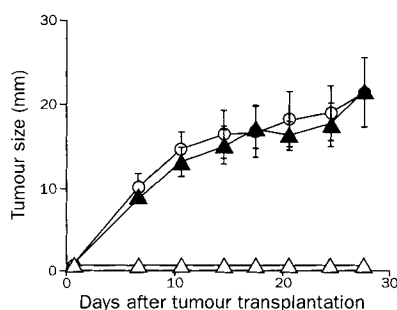
CD95 ligand in graft rejection

SIR — Bellgrau *et al.*¹ suggested that testicular allografts expressing functional CD95 ligand (CD95L) evade rejection by inducing apoptotic death of CD95-expressing recipient T cells activated in response to graft antigens. This implied that CD95L might be useful for creating immune-privileged tissue for a variety of transplant uses (see also ref. 2). We recently obtained a contradictory result indicating that CD95L expressed on the grafts induces a severe inflammatory rejection.

We transplanted a baby hamster kidney (BHK) fibroblast cell line, constitutively expressing transfected human CD95L complementary DNA, into nude mice lacking T lymphocytes. Xenogeneic BHK grew well in nude mice, but its CD95L transfectant was completely rejected (see figure). Administration of a neutralizing anti-CD95L monoclonal antibody³ reversed the rejection, indicating that it was induced by CD95L. This rejection is apparently independent of cytotoxic T lymphocytes and natural antibodies, as the rejection was also observed in mice with severe combined immunodeficiency, lacking both T and B lymphocytes. Similar rejections were observed when murine CD95L-transfected BHK was transplanted into nude mice and when human or murine CD95L-transfected murine lymphoma cell lines³ were transplanted into syngeneic or nude mice. *In vivo* depletion of natural killer cells, macrophages or granulocytes by administration of specific antibodies indicated that granulocytes are responsible for the rejection. Consistent with this was the massive infiltration of neutrophils observed in CD95L-expressing tumour grafts undergoing rejection.

Furthermore, CD95L-elicited peritoneal exudate neutrophils were strongly cytotoxic against the CD95L transfectants, and to a lesser extent against the nontransfectants *in vitro*. These results indicate that CD95L recruits neutrophils and activates their cytotoxic machinery, leading to acute graft rejection. Although the mechanism for the CD95L-mediated neutrophil recruitment and activation is unknown, it may involve the induction of interleukin-8 release from epithelial cells⁴ and the destruction of neutrophils⁵ by CD95L, resulting in local inflammation and non-specific graft damage. Nontransfectants were also rejected when transplanted together with the CD95L transfectants.

Bellgrau *et al.* used an allogeneic system, where graft rejection is mediated mainly by T cells, whereas we used a xenogeneic system, where rejection is mediated mainly by inflammatory neutrophils. However, this does not seem critical, as CD95L-expressing murine tumours were similarly rejected even in the syngeneic recipients. Bellgrau *et al.* used murine testis expressing murine



Rejection of CD95L transfectants. Parental BHK (○) or its CD95L transfectant (▲) were transplanted subcutaneously into nude mice which had received an anti-CD95L monoclonal antibody (▲) or control IgG (△).

CD95L, but we used hamster fibroblasts expressing human CD95L. It has been shown that human, but not murine, CD95L can be released in a functional, soluble form⁶, which may act chemotactically against neutrophils. This also does not seem critical, as murine CD95L transfectants were similarly rejected.

Bellgrau *et al.* transplanted grafts into the kidney capsule, whereas we performed subcutaneous grafts. It is well known that the site of transplantation greatly affects graft survival. The kidney capsule generally allows prolonged survival, as in the case of pancreatic islet grafts⁷; subcutaneous islet grafts are rapidly rejected even in syngeneic recipients⁸. The testicular allografts in the kidney capsule may not recruit neutrophils efficiently and may thus have evaded the neutrophil-mediated rejection. Alternatively, some factor other than CD95L, unique to testicular grafts, may be responsible for protection from neutrophils.

Neutrophil-mediated rejection induced by CD95L would severely limit its application for preventing graft rejection. Furthermore, CD95L is efficiently released from the transfectants³, which could lead to liver damage, as demonstrated by administration of anti-CD95 monoclonal antibody in mice⁹. These serious problems must be solved before CD95L can be used to make immune-privileged grafts.

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BELLGRAU *ET AL.* REPLY — The results reported by Yagita and colleagues are

unexpected in the light of recent findings published by our group and by Griffith and colleagues which indicate that CD95L expression by parenchymal cells in the testis and anterior chamber of the eye is required for the well-known immune-privileged status of these organs^{1,10}. One prediction of our results is that CD95L could be exploited to facilitate successful transplantation of a variety of tissues². This prediction has been borne out to a certain degree in that testis and the anterior chamber of the eye can protect allogeneic, as well as xenogeneic, tissues that do not express CD95L (for example, pancreatic islets) from graft rejection^{11,12}. In direct contrast to our results, the data provided by Yagita *et al.* suggest that CD95L expressed by transplanted cells would provoke graft rejection rather than protect against it.

Freshly isolated neutrophils, as opposed to lymphocytes and monocytes, express high levels of CD95, a type-I membrane protein that transduces an apoptotic signal, and to undergo apoptotic cell death in response to treatment with anti-Fas antibodies^{5,13}. A possible explanation of Yagita *et al.*'s results is that the CD95L-transfected tumour cells induced apoptosis of neutrophils, causing a nonspecific inflammatory response that destroyed CD95L-transfected as well as nontransfected tumour cells. Thus, one testable prediction is that *lpr* mice, which have neutrophils lacking functional CD95 expression, would be unable to reject CD95L-transfected tumours, in contrast to our preliminary results that testis tissue from BALB/c mice was rejected when transplanted under the kidney capsule of *lpr* mice¹.

Yagita *et al.* transplanted CD95L-expressing tumour cells subcutaneously, whereas we transplanted CD95L-expressing normal testis and Sertoli cells under the kidney capsule. The fate of CD95L-expressing testis and/or Sertoli cells transplanted subcutaneously is unknown, but preliminary results suggest that this site will not support engraftment of even syngeneic testis tissue (data not shown). Transplantation of various cells and tissues under the kidney capsule is thought to facilitate graft survival through more rapid vascularization than occurs subcutaneously. There is every reason to think that the kidney capsule in our system would contain as many neutrophils as the subcutaneous site chosen by Yagita *et al.*, especially considering the trauma associated with the transplantation procedure itself and the fact that Sertoli cells were transplanted in a fibrin clot.

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If CD95L-transfected tumour cells do induce neutrophil-mediated nonspecific tissue destruction *in vivo*, why are delayed-type hypersensitivity and other forms of cell-mediated immune reactions often devoid of neutrophils at the time that T cells mediating such reactions would express CD95L? It seems possible that the tumour cells used by Yagita *et al.*, in contrast to normal testicular, ocular or activated T cells, were responsible in part for the rejection response the authors observed by inducing neutrophil infiltration and/or activation. In this regard, granulocytosis is often observed as an accompaniment to neoplasia¹⁴⁻¹⁶.

Yagita and colleagues' findings are intriguing and will generate many further studies. Rensing-Ehl *et al.* recently reported that intraperitoneal administration of soluble CD95L in mice efficiently killed CD95-bearing lymphoma cells implanted in the peritoneal cavity without inducing hepatotoxicity¹⁷. Therefore, Yagita and colleagues' cautionary note that CD95 released from transfected tissue grafts might lead to liver damage could prove unwarranted in many transplant situations using CD95L-transfected tissue. Ultimately, the usefulness of CD95L in preventing graft rejection must be tested by expressing the molecule in normal tissues, such as pancreatic islets.

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Drought tolerance in tobacco

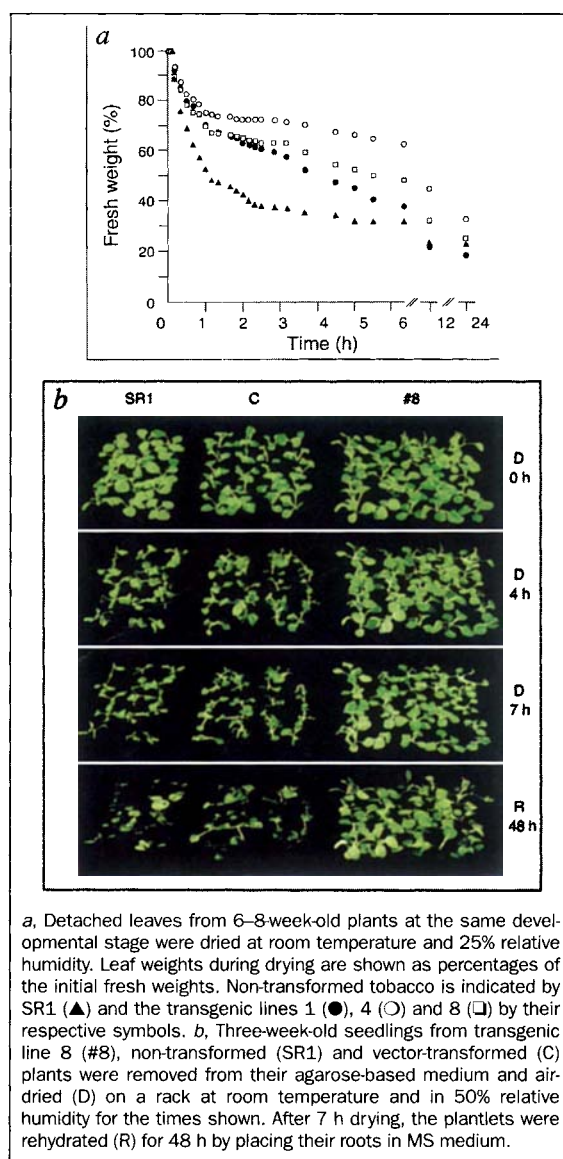
SIR — Water stress due to drought, salinity or freezing is the main limiting factor for plant growth and distribution^{1,2}. A common response to water deficit is the accumulation of osmoprotectants such as sugars and amino acids^{2,3}. High concentrations of the osmoprotectant trehalose occur in many anhydrobiotic organisms that survive complete dehydration³ and, although rare in higher plants, this nonreducing disaccharide can be found in some resurrection plants⁴. Trehalose also efficiently stabilizes dehydrated enzymes and lipid membranes *in vitro*^{3,5}. Here we show that engineering the synthesis of small amounts of trehalose in tobacco greatly increases the plant's ability to survive drought.

The gene encoding the trehalose-6-phosphate synthase subunit (TPS1) of yeast trehalose synthase⁶ was spliced to the promoter of the Rubisco small subunit gene, *ats1A*, from *Arabidopsis thaliana*, and introduced to tobacco by *Agrobacterium*-mediated gene transfer⁷. The green tissue (but not the roots) of 20 out of 26

kanamycin-resistant transformants contained a polypeptide of relative molecular mass 56,000, recognized by anti-serum to TPS1 (ref. 6). Leaf extracts from transgenic line 4 catalysed the synthesis of trehalose-6-phosphate from glucose-6-phosphate and UDP-glucose (3.1 ± 0.44 U per g dry leaf), whereas non-transformed SR1 and vector-transformed control plants had negligible activity (≤ 0.3 U per g dry leaf).

Trehalose, identified by its chromatographic behaviour and degradation by a specific trehalase, was present in the leaves, flower buds and even roots of TPS1-positive transformants. Leaves from 7 lines (lines 1, 4, 5, 6, 8, 16 and 19) grown in a greenhouse contained 0.8–3.2 mg trehalose per g dry weight, in contrast to 0.06 mg per g in non-transformed or vector-transformed plants. Trehalose-6-phosphate was not found in trehalose-producing lines, suggesting that tobacco dephosphorylates this product of TPS1. Trehalose accumulation caused no obvious morphological changes in self-pollinated progeny of TPS1-positive plants, but decreased the growth rate by 30–50% in conditions optimal for control plants. This may be avoidable by using stress-activated promoters⁸ to drive TPS1.

When detached leaves were air-dried, those from trehalose-producing plants reproducibly lost water more slowly than control leaves and remained fresh even after 24 h of drying (*a* in the figure). Three-week-old TPS1-positive seedlings, obtained by self-pollination of line 8, were subjected to air-drying with non-transformed and vector-transformed control plants (*b* in the figure). The control seedlings showed signs of wilting after 2 h of drying and totally collapsed after 7 h; TPS1-positive seedlings were only marginally affected by this treatment. After rehydration, the trehalose-producing plants recovered turgor and recommenced growth, while the controls died. Similar differences in drought tolerance were observed in 4-month-old *in vitro*-propagated plants.



a, Detached leaves from 6–8-week-old plants at the same developmental stage were dried at room temperature and 25% relative humidity. Leaf weights during drying are shown as percentages of the initial fresh weights. Non-transformed tobacco is indicated by SR1 (▲) and the transgenic lines 1 (●), 4 (◻) and 8 (◻) by their respective symbols. **b**, Three-week-old seedlings from transgenic line 8 (#8), non-transformed (SR1) and vector-transformed (C) plants were removed from their agarose-based medium and air-dried (D) on a rack at room temperature and in 50% relative humidity for the times shown. After 7 h drying, the plantlets were rehydrated (R) for 48 h by placing their roots in MS medium.

Production of trehalose seems to improve the drought tolerance of tobacco. This is consistent with recent reports indicating that the accumulation of other osmoprotectants, such as mannitol⁹, fructan¹⁰ or proline¹¹, may increase tolerance to water deficit. In apparent contrast to the tolerance afforded by mannitol production⁹, which enhances growth under stress applied during a narrow developmental window, protection by trehalose is seen in both young seedlings and older plants. The trehalose concentrations (≤ 5 mM in the cytosol) seem too small for osmotic adjustment, so stabilization of cellular structures and macromolecules by trehalose may underlie both the improved water retention and enhanced desiccation tolerance.

These results demonstrate a marked,

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heritable enhancement of drought tolerance of both intact plants and detached leaves. Engineering trehalose biosynthesis into other plant species may provide crucial protection against frost and salinity, as well as drought, and confer improved storage properties after harvest.

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Insect pheromone in elephants

SIR — (Z)-7-dodecen-1-yl acetate is used by the females of more than 126 species of insects, especially Lepidoptera, as part of their pheromone blends to attract insect males¹. Female Asian elephants, *Elephas maximus*, also use a pheromone to signal to males their readiness to mate². This pheromone is released in their urine during oestrus and before ovulation. We have now isolated this compound by bioassay-guided fractionation and purification. Remarkably, it is the same compound, (Z)-7-dodecen-1-yl acetate, used by insects including the cabbage looper, tomato looper, silver Y and turnip moths, the dingy cutworm, and the sugarcane stalk borer¹.

Female Asian elephants have a uniquely long oestrous cycle³. Urine from pre-ovulatory females elicits a high frequency of flehmen responses (a distinct truncal movement that facilitates chemosensation via the vomeronasal organ) from Asian bull elephants⁴. During this chemosensory response, liquids are transported by the trunk tip finger to the paired openings of the ducts of the vomeronasal organ in the anterior region of the hard palate. We have used this discrete motor pattern, which is exhibited specifically during the sensory evaluation of chemicals, to develop a specific and quantitative bioassay for isolating pheromone from oestrous urine⁴.

Five sexually mature male Asian elephants (age 12–34 yr), four of whom were experienced breeders, were tested, principally at the Metro Washington Park Zoo, Portland, Oregon. Samples were dissolved in acetone in 500 ml of water. The number of check, place and flehmen responses in a defined period were recorded (see table). Physiological concentrations of the identified pheromones were estimated based on biological activity of oestrous urine aliquots, calculated

BIOASSAY RESPONSES PER HOUR OF FIVE MALE ASIAN ELEPHANTS

Test elephants* (no. of trials)	Concentration (mM) [†]	Median (25–75 percentile)		
		Check	Place	Flehmen
Male 2 [§]				
(8)	0.5 mM	6.0 (2.5–9.5) [§]	0 (0–0.75)	0 (0–15)
(7)	1.0 mM	8.0 (4.8–9.8) [§]	2.0 (0.25–2.0) [§]	1.0 (0.25–2.0) [§]
(3)	2.0 mM	9.0 (2.3–21.0)	3.0 (0.75–3.0) [§]	1.0 (1.0–3.3) [§]
(2)	5.0 mM	6.5 (4.0–9.0) [§]	5.5 (0–11.0) [§]	1.5 (1.0–2.0) [§]
(5)	2.0 mM [†]	15.0 (3.0–21.0) [§]	15.0 (6.5–22.0) [§]	17.0 (2.8–25.0) [§]
Males 1–5	Pre-ovulatory urine	7.0 (5.0–8.0) [§]	5.0 (4.0–8.0) [§]	7.0 (1.4–9.2) [§]
Males 1–5	7-DDA	6.0 (4.0–9.0) [§]	6.0 (4.0–10.0) [§]	4.0 (2.0–6.0) [§]
Controls (10)				
Females 1–5	2.0 mM	0 (0–0.75)	0 (0–0.25)	0 (0–0)
Males 1–5	<0.5 M acetonitrile	0 (0–0)	0 (0–0)	0 (0–0)
Males 1–5	<0.5 M acetone	0 (0–0.25)	0 (0–0)	0 (0–0)
Males 1–5	<0.2 M dichloromethane	0 (0–0)	0 (0–0)	0 (0–0)
Males 1–5	<0.005 M hexanal	0 (0–0.75)	0 (0–0.25)	0 (0–0)
Males 1–5	Non-oestrous urine	0 (0–1.0)	0 (0–0.5)	0.5 (0–1.0)

[†]'Check' responses occur when the dorsal trunk tip finger comes into direct contact with liquid samples. 'Place' responses occur when the entire trunk tip is placed for several seconds in liquid samples. These responses occur before and may be either independent or an integral part of the flehmen response. Samples eliciting check responses often elicit flehmen responses with increasing concentrations.

[§]Elephants participating in the assays live at Metro Washington Park Zoo, Portland, Oregon (3 males, 5 females); at Busch Gardens, Tampa, Florida (one male and 5 females, courtesy of R. Schmitt); and at Dicksen Park Zoo, Springfield, Missouri (one male).

[†](Z)-7-dodecen-1-yl acetate (7-DDA) with ~2% E isomer in 0.5 M acetone in 500 ml water.

[†]In non-oestrous urine instead of water.

[§]Similar results were obtained from all 5 test animals.

[§]Significant differences from controls. Data are expressed as medians (25–75 percentile). Analyses and tests for significant differences ($P < 0.05$) included Kruskal–Wallis one-way ANOVA on ranks, Mann–Whitney rank sum tests, Dunn's test and Wilcoxon rank sum test.

[†]Penile erections observed.

bioassay units⁴ and quantitative gas chromatography. Bioassay controls included non-oestrous female urine, solvents used for extraction and purification, and various synthetic compounds. When new compounds are tested, they occasionally elicit a moderately high frequency of flehmen responses which disappear in later assays. Multiple bioassays were conducted for each sample concentration to distinguish the sustained positive responses characteristic of the pheromone from such 'novel substance' responses.

Urine was collected from nine mature female Asian elephants during pre-ovulatory days, determined from serum progesterone concentrations, assessment of cervical mucus, and daily monitoring of responses of males to females³. The purification procedure used a series of fractionation steps⁴. The active fraction finally obtained was identified as (Z)-7-dodecen-1-yl acetate (97%) and (E)-7-dodecen-1-yl acetate (3%) using gas chromatography/mass spectrometry, magnetic resonance spectrometry and appropriate reaction chemistry⁵ (data not shown).

The biological activity of (Z)-7-dodecen-1-yl acetate was confirmed using commercially available synthetic material ((Z)-7-dodecen-1-yl acetate with about 2% E isomer; Sigma) using five bulls at three sites (see table). Robust positive responses were obtained with no decrease in the frequency of flehmen responses in successive bioassays (see table). The bioassay results of both elephant-derived and authentic (Z)-7-dodecen-1-yl acetate in water compared with urine suggests that additional components in urine, possibly protein-

aceous, confer additional specificity.

That elephant and insect females use an identical molecule to signal males of their readiness to mate is an outstanding example of convergent evolution capitalizing on the chemical properties of such a compound, presumably its volatility. Future inter-species tests will include both the possible efficacy of this pheromone with male African elephants and the effect on local insect species populations known to utilize this pheromone. We now aim to define the source of the pheromone and its signal transduction function in the vomeronasal organ.

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Beyond reasonable doubt

Tim Crane

Soul-Searching: Human Nature and Supernatural Belief. By Nicholas Humphrey. Chatto and Windus: 1995. Pp. 244. £18.99.

In 1991, Darwin College, Cambridge, was given a substantial bequest to fund a research post in parapsychology. The event became something of a *cause célèbre*. Various academics at the university objected to accepting the money: D. H. Mellor, a professor of philosophy, remarked on BBC radio that funding such a position would be like funding a research post to establish whether the Earth is round. Other members of the college were (understandably, perhaps) reluctant to turn down any offer of money for research. In the end the situation was resolved to the satisfaction of the sceptics: Nicholas Humphrey, a psychologist and broadcaster, was given the post to carry out research into why people believe in parapsychology. This book is one result.

Was the money well spent? *Soul-Searching* is a lively, entertaining book, packed with pointed quotations from literary and scientific sources. The upshot of Humphrey's research comes as no surprise: the reason people believe in parapsychological phenomena is because they want to believe in them, because they want to think there is something more to human beings than just the matter comprising them. Belief in parapsychology is, like belief in the immortality of the soul, wishful thinking deriving from our inability to accept the brute facts of our material existence.

This conclusion, although not in itself implausible, does not seem to be the product of extensive empirical research. Little of this book is the result of, say, opinion polls or interviews with parapsychologists, and Humphrey does not quote very often from the works of parapsychologists. It is hard to avoid speculating that Humphrey knew what the outcome of his research would be even before he started.

But this is not in itself a criticism of the book, because Humphrey has set himself a larger task than simply explaining why people believe in parapsychology: the task of explaining why it is irrational to believe in it. Humphrey points out that there is a strange paradox at the heart of the parapsychological research programme. On the one hand, parapsychologists claim that they are just open-minded scientists trying to account for recalcitrant 'psi-phenomena'. But on the other hand, parapsychologists often appear convinced (before doing their research) that paranormal phenomena must, in John Beloff's

words, "forever defy scientific analysis". And therein lies the peculiarity of the approach: success in the science of parapsychology amounts to discovering things that cannot be explained by science.

Humphrey is good at teasing out the contradictions and tensions in parapsychologists' claims. He also does a good job of underlining the curious (and inexplicable) banality of the supposed psi-phenomena: why can psychic powers bend spoons but not prevent air crashes? Less convincing is his insistence that a belief in an immaterial mind requires a commitment to the paranormal. For there are many ways of denying materialism — the vague philosophical doctrine that everything is material — without embracing parapsychology. And it would also be possible to hold (although few parapsychologists do) that psi-phenomena will ultimately be explained in terms of physical forces and laws. Materialism, then, is irrelevant: the issue is not whether parapsychological phenomena are inconsistent with materialism, but whether they are inconsistent with the facts, whatever these may be.

The right way to approach claims about parapsychological phenomena, it seems to me, is David Hume's. In his essay "Of

Miracles" (1777), Hume argued that, given a report of a miracle, it is in general more reasonable to believe that someone somewhere is deluded than it is to believe that some fundamental law of nature has been disrupted. An advantage of this approach is that sceptics do not have to explain how the 'miracle' occurred, and nor do they have to prove them impossible. Rather, given that there is an explanation consistent with the laws of nature (in terms of the credulity and fallibility of people), the apparent miracle can be dismissed.

Humphrey is fully behind Hume here. But towards the end of the book he raises the stakes in the argument against parapsychology and attempts to argue not only that parapsychological phenomena are improbable given what we know, but also that they are logically impossible. That is, the claim is not just that psi-phenomena do not happen: it is the stronger claim that they cannot happen. This seems to me to be a mistake. It is one thing to say that something cannot happen given what we know about the world, but quite another to say that it cannot happen in any sense at all. The fact that we can imagine extrasensory perception and the like is evidence for the fact that they are possible in some sense; and the sceptic should not have to deny this. As Humphrey's book shows, the case against parapsychology is strong enough without its having to establish the absolute impossibility of something that simply does not happen. □

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FLOWER power — Joseph Dalton Hooker in the Himalayas (c.1849), accepting rhododendrons as a colonial tribute. He believed that imperial territories should look to the mother country for scientific guidance. Picture taken from *Cultures of Natural History* edited by N. Jardine, J. A. Secord and E. C. Spary, which contains 24 essays on the history of natural history from the sixteenth to the late nineteenth century. Cambridge University Press, £65, \$89.95 (hbk); £22.95, \$29.95 (pbk).

Decisions spared

R. V. Jones

Hitler's Uranium Club: The Secret Recordings at Farm Hall. Edited by Jeremy Bernstein. *American Institute of Physics*: 1995. Pp. 427. £25.

FOR the last six months of 1945, ten German physicists were held in isolated but gentle detention at a country house, Farm Hall, some 20 miles northwest of Cambridge in England. They had been taken there, following the collapse of Germany, because they were believed to have been concerned in the exploitation of nuclear fission as a means of making an 'atomic' bomb. Unknown to them, their conversations were monitored and recorded because of the light they might throw on how near the Germans had come to making a bomb.

Transcripts of the conversations were regularly sent under highest secrecy to the authorities responsible for nuclear development in the United Kingdom and the United States. These transcripts, extracts from which were quoted by General Leslie Groves and Samuel Goudsmit in their subsequent books, were kept secret until 1992, when the Lord Chancellor (Lord Mackay), at the request of representatives of the Royal Society and the British Academy, ruled that they could at last be made public.

It was further decided that the full transcripts should be published (and translated where necessary) by the Institute of Physics in the United Kingdom with an introduction by F. C. Frank, who had not only read the transcripts at the time they were made but had also visited the detainees at Farm

Hall and had known some of them in Germany before 1939. The resulting book was published by the Institute of Physics in 1993 under the title *Operation Epsilon: The Farm Hall Transcripts*, and the University of California Press sold and distributed it in the United States (for a review see *Nature* 364, 114; 1993).

Frank's introduction and comments have so much authority that there hardly seems need for a second book on the same subject; but this is what we now have in Jeremy Bernstein's *Hitler's Uranium Club*, which gives the same transcripts but with an extended commentary for readers who do not have the "considerable technical background" that Bernstein deems

mates of the necessary amount of uranium-235 suggested that it was far beyond anything they could hope to achieve*. This is what British and American physicists would also have decided, had it not been for the vital contribution of Rudolf Peierls and Otto Frisch in Birmingham, who showed that the required amount of uranium-235 might be within the bounds of feasibility. The effort needed, although Herculean, was successfully achieved by American engineers under the leadership of General Groves.

The question has often been asked: why did the Germans not succeed in making an atomic bomb? It would be far too crude to reply that this was because they had neither

Peierls and Frisch, nor Groves. But it is worth remarking that Peierls and Frisch had both come to England as "birds of passage" in flight from the Nazis. And it is also worth remarking that the motivation in the United Kingdom was far greater than that in Germany. In 1940, I can recall James Tuck in the Cabinet Office saying to me that the atomic bomb, if only we could make it, seemed our one hope of winning against Germany.

On the other side, at least until 1942, there was no such desperation in Germany, and there seemed no need for German physicists, even those

who wanted to win the war, to question Heisenberg's estimate that pointed to the unfeasibility of bomb production in the foreseeable future. And although the alternative approach to a bomb through plutonium had been suggested by both Carl Friedrich von Weizsäcker and Fritz Houtermans, this was not followed up; and the most that was done after 1942 was towards a source of heat for a "boiler".

Naturally, as they recovered from their first shock, the Farm Hall detainees began to ask why they had failed so signally. Reproaches went so far as Otto Hahn (who had refrained from working towards a bomb) saying to Heisenberg "At any rate, Heisenberg, you're just second-raters and you may as well pack up", to which Heisenberg replied "I quite agree". Later in the same discussion, von Weizsäcker offered: "I believe the reason we didn't do it was because all the physicists didn't want to do it on principle. If we had all wanted Germany to win the war we could have succeeded."

This comment could readily be construed as 'sour grapes', but it could have contained a pip of truth in that there was no such intense motivation in Germany as had led, for example, to microwave radar



Farm Hall as it looked in March 1987.

necessary for a full appreciation of both the transcripts and Frank's introduction.

What Bernstein has therefore provided is a running commentary on the transcripts themselves, supplemented by sketches of the historical, scientific and social backgrounds to the statements made by the detainees to one another. He has added further interest by including a diary by one of the detainees, Erich Bagge, and letters from another, Max von Laue, to Paul Rosbaud. The transcripts provide a unique insight into the characters, relationships and thoughts of a remarkable group of individuals, nearly all deeply involved in nuclear developments in wartime Germany, at the very time when they were told the news of the bomb on Hiroshima.

They were stunned — in Werner Heisenberg's case so much that in estimating the amount of uranium-235 that must have been needed for a bomb, he could at first resort only to a 'back of the envelope' calculation pathetically cruder than any he may himself have made earlier in the war. Within a few days, though, he had recovered sufficiently to produce a better understanding of what the Allied physicists had achieved.

The transcripts show that the Germans had given up the prospect of making a bomb in 1942, mainly because their esti-

A Bedside Nature

Heisenberg's account of Germany's failure to produce a nuclear bomb is one of many contributions about science and scientists during and after the war that appear in the recently published *A Bedside Nature: Genius and Eccentricity in Science 1869–1953* edited by Walter Gratzer with a foreword by Stephen Jay Gould. This paperback anthology "presents a panorama of science seen against the backdrop of nineteenth- and twentieth-century history, with its triumphs, débâcles, surprises and absurdities". Macmillan, £19.95, \$29.95 (nonsubscribers); £14.95, \$24.50 (subscribers). Further details available from *Nature* on +44(0)171 843 4962 (telephone).

*See for example I. M. Klotz's review of *Nazi Science* by Mark Walker in *Nature* 379, 410 (1996).

in the United Kingdom.

Von Weizsäcker's comment was afterwards so elaborated by Robert Jungk in *Brighter Than a Thousand Suns* as to suggest that German physicists in contrast to their Allied counterparts had "kept their hands clean" and intentionally refrained on the grounds of conscience from working towards a bomb. No doubt there were some individuals such as Hahn who would have so refrained had they been asked to help make a bomb, just as there were others who, as patriots, would have joined a bomb project with enthusiasm. In these matters German physicists would have been hardly less divided than their Allied counterparts had the choice faced them.

The transcripts for all their interest and the insight they provide give little grounds for disputing what Heisenberg himself said (*Nature* 160, 214; 1947 — see box on previous page): "In the upshot they [the German physicists] were spared the decision whether or not they should aim at producing atomic bombs". What, though, the transcripts do provide, along with Frank's introduction no less than Bern-

stein's commentary, is enlightenment as to why the German physicists were spared the decision: it was too far beyond their technological vision in the conditions of the war.

To complete this review on a less sombre note, let me recall that the Farm Hall detainees were concerned about their future in Germany and whether they would be allowed to return to the pursuit of physics. The same question, of course, had been considered by the Allied authorities. I was present at an Anglo-American conference of military men and scientists when we decided that research in pure science should be allowed in Germany, but that applied research should be restricted. An American colonel then asked how we were to define 'pure' and 'applied'. After several of us had volunteered definitions that left the colonel confused, he said: "I see, we should define 'pure research' as 'research with no known objective!'". □

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Apocalypse of our own making

Bernard Wood

The Day Before Yesterday: Five Million Years of Human History. By Colin Tudge. Cape/Scribner's: 1996. Pp. 390. £18.99, \$27.50. Published in the United States as *Time Before History: Five Million Years of Human Impact*.

Dominion: Can Nature and Culture Co-Exist? By Niles Eldredge. Holt: 1995. Pp. 190. \$25.

THE 'rules' of the evolution game are well-known. The winners are the 'fittest', the losers are the 'less fit'. What makes the game so difficult for the players are two devilish twists to the rules. The first is that the criteria for fitness are in a state of flux — they are constantly being redefined. The second is that the nature of the competition is partly defined by who the competitors are. The only consolation to the participants is that all the players are equally likely to be disadvantaged.

That is until about 10,000 years ago when one of the competitors stumbled on 'culture'. Culture was like the introduction of the carbon-fibre racket into tennis. It enabled the average player to stay in the competition longer. But although it meant swift elimination for a few competitors, for a long time most players could stay in the game. Developments from this thing we call culture are, however, now beginning to threaten the survival of the game itself. Can culture find solutions fast enough to

the problems it creates, or should we forgo our cultural advantage for the benefit of all the players, ourselves included? There is little point in winning if we cannot survive to enjoy it. These are the broad themes explored in these two books.

One of the recent great achievements of science has been our deepening, but by no means comprehensive, understanding of past climates. Palaeoclimatology demonstrates the essential continuity between the biological and the physical sciences. It is, and always will be, impossible to 'prove' that climate was crucial in shaping evolutionary history, but there is a lot of circumstantial evidence to suggest that it was. If the rate of evolutionary change depended on some internal mechanism, why did a wide range of mammals adjust the way in which they processed their food at roughly the same time? It is more parsimonious to suggest that they were responding in different, but analogous, ways to an extrinsic stimulus, and the prime suspect is climate.

Although most of us are aware that it is the Earth's rotation that takes us away from the Sun's rays at night and towards them at dawn, fewer people appreciate the crucial influences on our climate of cycles caused by changes in the axis of that rotation and by deviation from circularity (or eccentricity) of the Earth's orbit around the Sun. These influences have been superimposed on a 40-million-year-old process of global cooling linked to the

buckling in the Earth's crust caused by continental movements.

As Colin Tudge shows, the increasing influence of two of these cycles combined with the cooling effect of the inexorable rise of the Tibetan plateau seems to have triggered some three million years ago the most recent acceleration of the trend towards cooler, drier climates. A million years ago, this trend culminated in the increasing dominance of the roughly 100,000-year eccentricity-driven cycles leading to regular episodes of extreme cooling. The 'savannah' hypothesis of human origins, in which the cooling begat the savannah and the savannah begat humanity, is now discredited, but there is compelling evidence that global climate change combined with local uplift was the trigger that eventually resulted in the emergence in Africa of culture-bearing hominids. So it is high irony that modern humanity is now threatening the viability of the global ecosystem through its effects on climate.

Tudge sets the events of human evolution in context. His knowledge of the evolutionary changes in the nonhominid components of the contemporary fauna is impressive and he is an equally effective guide to the mechanisms of human-induced climate change. Some may find his tendency to explore themes tangential to the main argument irritating, but this is more than compensated for by his ability to convey his own excitement about the elegance of much of the science involved, and he packs in a great deal of information.

Niles Eldredge could hardly offer a greater contrast in writing style. He could never be accused of being prolix, but readers may find his style so terse that the significance and meaning of parts of the text may elude them. His interpretation of the hominid fossil record does not always accord with my own understanding, but this does not detract from the burden of his argument. This is a 'single-issue' book that wastes no time on frills.

The message of both books is that the potency of modern technology is such that humanity now has the means to modify climate instead of being modified by it. We accord ourselves the status of being the 'first' of all the creatures on Earth. The terrible truth is that we are the first creatures to have the means to destroy habitats on a global scale. Our only hope is that our intellect will call a halt to our folly in good time. For this we need to redefine success as being long-term survival rather than short-term riches. We must all hope that our collective intelligence will rise to the challenge. □

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CATFISH migration in the Teotônio rapids of the upper Rio Madeira in the Amazon Basin. Some species migrate 2,000–3,000 km between the estuary and headwaters where they feed and spawn. The Amazon, the world's largest rainforest and river ecosystem, is rapidly being transformed by the fishermen, ranchers, gold miners and others seeking to tap the resources of the region. In the past five decades, the rivers and their floodplains have undergone more

environmental change than in all of human history. This change and its global implications are explored in *Floods of Fortune: Ecology and Economy Along the Amazon* by M. Goulding, N. J. H. Smith and D. J. Mahar. The authoritative text is accompanied by stunning photographs illustrating the wetland's wildlife as well as the complex environmental and social problems associated with its development. Columbia University Press, \$29.95.

To coldly go

John Haslett

Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences. Edited by F. S. Chapin III and C. Körner. Springer: 1995. Pp. 332. DM198, £92.50, \$143.

"LIFE in the Freezer", a recent television series about Antarctic organisms, showed some of the more spectacular aspects of nature under the extreme conditions of cold and desiccation. Predictably, it mainly featured penguins, seals and whales, no doubt because of their aesthetic appeal. But because penguins are found only in the Southern Hemisphere, and seals and whales are not generally found on mountains, the editors of this book on organisms in similarly inhospitable arctic and alpine environments were faced with a formidable task in attracting a wide audience, albeit within scientific circles. The bait they have used is the theme of 'biodiversity', which serves as the framework for the book. The approach is both appropriate and useful.

Biodiversity is more than simply a measure of the number of species; it describes the variety of life over a wide spectrum of levels, from genetics through the taxonomic and ecological hierarchies to communities, habitats and ecosystems. Human activity is causing serious destruction of biodiversity at all of these levels. As our awareness of the problem has

increased, two main areas of concern have become apparent. The first is that we are losing biodiversity faster than science can catalogue it, so attention is centred on tropical 'hotspots' — regions extremely rich in species, many of which remain undescribed. In this way, biodiversity has become synonymous with (tropical) species richness, but that area is only part of the story. The second area of concern, and the one that provides the book's focus, is ecosystem function: if we lose variety within a system, will the system be able to keep going in a similar way? Here the idea of 'keystone' species (or other functional ecological units) becomes important, but their identification requires knowledge of the patterns and causes of the present biodiversity of the ecosystem.

Arctic and alpine ecosystems are characteristically low in species, to the extent that they are often referred to (incorrectly) as 'simple' ecosystems. Yet, like the species-rich tropics, they are also prone to heavy losses in biodiversity through human activity. The book serves as a timely reminder of this vulnerability.

Chapters provide a basis for discussion of arctic and alpine biodiversity at all levels, from genetics to ecosystem, although there is clearly some confusion among the authors as to the precise meaning of 'biodiversity'. The three main sections reflect the principal objectives of the editors: description of the patterns and causes of diversity, development of a framework for predicting change and discussion of the results of change. Ambitious indeed; and, viewed in these terms, the book is only

partly successful. Although the standard of science is generally high, there is an overall lack of balance in subject matter. More than two-thirds of the contributions are specifically about arctic environments, and a similar proportion are devoted to plants. If, like me, you happen to have an interest in animals on mountains, a single chapter is your lot. But it is pleasing to find discussion of microbial biodiversity, a topic often neglected by ecologists.

Another difficulty is failure to take proper account of the differences between arctic and alpine ecosystems. Alpine environments are extremely varied over a wide range of spatial scales, with correspondingly complicated ecological dynamics. This makes them unique. So it is wrong, as has traditionally been the case, to lump 'alpine' environments with 'arctic' environments simply because they are both extremely cold. It is also wrong to attempt to extrapolate across the arctic-alpine divide when there are no good grounds for doing so, a temptation even the editors cannot resist in their final summary chapter.

Nevertheless, the book is important: appreciation of biodiversity in habitats characteristically low in species is crucial to our understanding of the global biodiversity 'crisis'. But before we can think of predicting and discussing the consequences of change in biodiversity, we need to think more about the differences between ecosystems, not just their similarities. □

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Similar rates of modern and last-glacial ocean thermohaline circulation inferred from radiochemical data

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Today, the ocean thermohaline circulation transports half of the ^{231}Pa produced by radioactive decay in the Atlantic Ocean water column to the Southern Ocean. This export respectively imparts low and high $^{231}\text{Pa}/^{230}\text{Th}$ ratios to the surface sediments of these oceans. Ocean sediments from the Last Glacial Maximum bear a similar isotopic fingerprint, implying that advection of North Atlantic Deep/Intermediate Water into the Circumpolar Deep Water of the Southern Ocean occurred at a similar—or slightly higher—rate during the last glacial period.

THE broad features of modern ocean circulation have been described as a thermohaline-driven conveyor belt (Fig. 1a) initiated by the formation of North Atlantic Deep Water (NADW). This general pattern of circulation affects global climate by transporting heat from the subtropical North Atlantic to high northern¹ and southern² latitudes and by influencing the partitioning of CO_2 between deep water and the atmosphere^{3,4}. Past changes in the mode of deep-water circulation could thus have played an important role in late Quaternary climate cycles. Down-core variations in the $\delta^{13}\text{C}$ (refs 5–7) and Cd/Ca ratio^{8,9} of benthic foraminifera shells imply significant changes in deep-water circulation during the Last Glacial Maximum (LGM). Both tracers have documented a vertical redistribution of nutrients in the glacial Atlantic, suggesting the replacement of NADW by a shallower Glacial North Atlantic Deep/Intermediate Water (GNAIW). In the Southern Ocean, however, where NADW mixes with recirculated Indo-Pacific Deep Water (IPDW) to form Circumpolar Deep Water (CPDW), the two tracers disagree^{10,11}. If both are taken as reflecting deep-water nutrient concentration, the 1‰ decrease in benthic $\delta^{13}\text{C}$ during the LGM is incompatible with the nearly constant Cd/Ca ratios. The carbon isotopes imply a higher nutrient concentration in glacial CPDW compared to IPDW, and can be interpreted as reflecting formation of North Pacific Deep Water^{5,12} and a total shutdown of the global conveyor belt circulation^{13,14}. In this model (Fig. 1B, a), GNAIW forms an internal loop ventilating Atlantic intermediate and upper deep waters without significant export of water into CPDW and the Pacific Ocean. Alternatively, the Southern Ocean benthic $\delta^{13}\text{C}$ record is also consistent with a glacial conveyor belt, initiated by GNAIW, that exported water directly into the Indo-Pacific (Fig. 1B, b), bypassing an isolated, nutrient-enriched CPDW¹⁵. In contrast, Cd/Ca ratios imply that glacial CPDW nutrient concentration remained intermediate between Atlantic and Pacific deep water during the LGM, and that Atlantic water was continuously exported into CPDW, as part of the GNAIW-driven glacial conveyor belt^{10,16} (Fig. 1B, c).

Although progress has recently been made towards resolving the discrepancy between the benthic $\delta^{13}\text{C}$ and Cd/Ca records of the Southern Ocean^{17,18}, it is clear that palaeonutrient proxies cannot unambiguously resolve whether there was effective export

of deep or intermediate water from the Atlantic during the LGM. We thus propose to address this issue using a different approach in which we compare the ratio of two particle-reactive nuclides (^{231}Pa and ^{230}Th ; generated in sea water by the decay of dissolved uranium) in Holocene and LGM Atlantic sediments. Our method is based on the observation that, as a result of modern thermohaline circulation, about 50% of the ^{231}Pa and about 10%

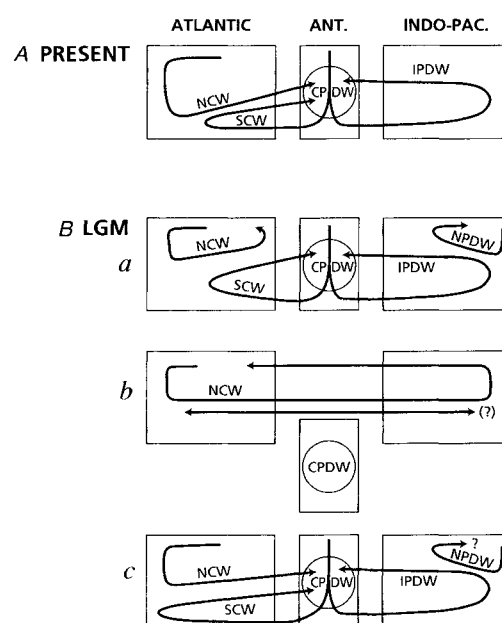


FIG. 1 Diagrams of deep-water circulation between the Atlantic, Antarctic and the Indo-Pacific oceans at present (A) and during the Last Glacial Maximum (LGM; B). The three hypothetical circulations for the LGM are based on conflicting palaeonutrient data^{9–11,15}. NCW, Northern Component Water, that is present-day North Atlantic Deep Water (NADW) or glacial North Atlantic Deep/Intermediate Water (GNAIW); SCW, Southern Component Water; CPDW, Circumpolar Deep Water; IPDW, recirculated Indo-Pacific Deep Water; NPDW, North Pacific Deep Water.

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of the ^{230}Th produced in the Atlantic water column are advected into the Southern Ocean. This export imparts comparatively low $^{231}\text{Pa}/^{230}\text{Th}$ ratios to the Atlantic Holocene sediments and high ratios to sediments deposited in the Southern Ocean. Decay-corrected sedimentary $^{231}\text{Pa}/^{230}\text{Th}$ ratios averaged over the entire Atlantic thus constrain the average residence time and export flux of Northern Component Water (NCW; that is, NADW or GNAIW), independently testing the validity of the different palaeonutrient-based LGM circulation models (Fig. 1). The same ratio in Southern Ocean sediments indicates whether deep/intermediate Atlantic water is exported into or bypasses the CPDW. We found a ^{231}Pa deficit in glacial Atlantic sediments as pronounced as for the Holocene, implying continuous export of NCW from the Atlantic Ocean. In addition, high $^{231}\text{Pa}/^{230}\text{Th}$ ratios in glacial Southern Ocean sediments indicate that NCW reached CPDW. These results thus support the glacial circulation model where vigorous Glacial North Atlantic Deep/Intermediate Water exported Atlantic water into Circumpolar Deep Water (Fig. 1*b, c*).

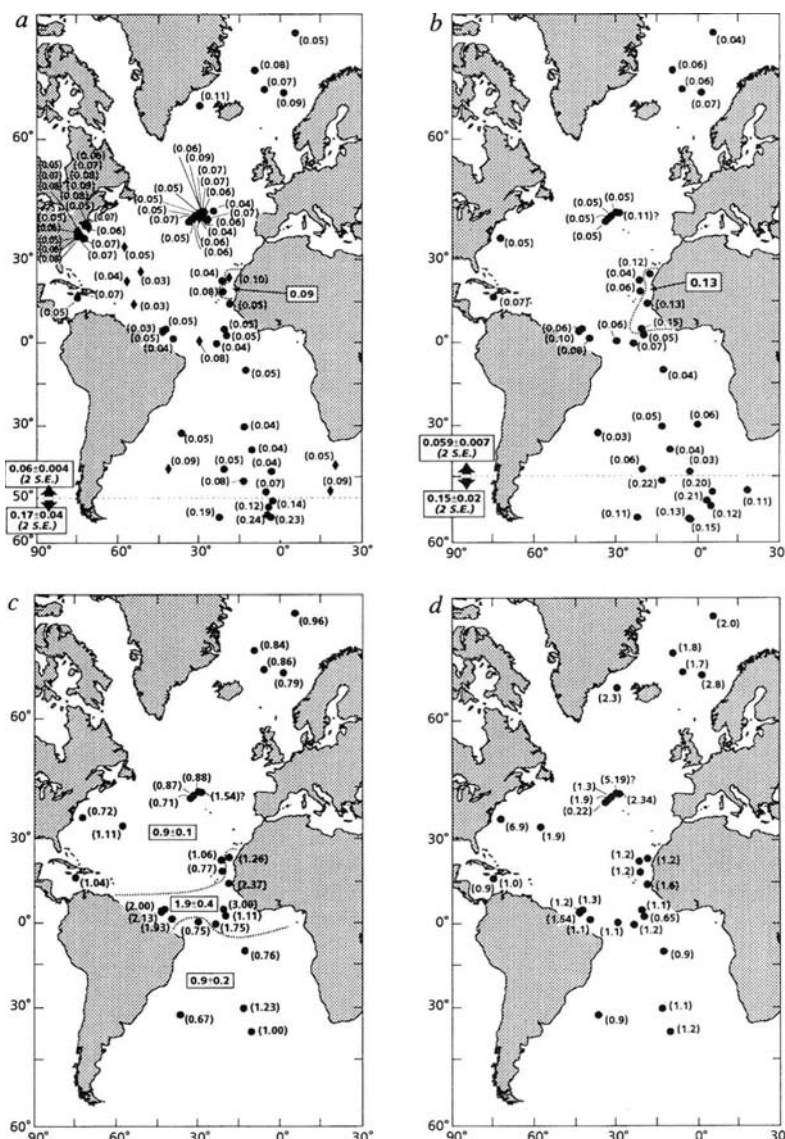
Oceanic fractionation of ^{231}Pa and ^{230}Th

^{231}Pa (half-life $t_{1/2} = 3.2 \times 10^4$ yr) and ^{230}Th ($t_{1/2} = 7.5 \times 10^4$ yr) are both produced in the water column from α -decay of U isotopes dissolved in sea water (^{235}U and ^{234}U , respectively). Because U

essentially behaves conservatively¹⁹, the rate of production (β) of these two radionuclides is uniform throughout the water column: $\beta_{\text{Th}} = [^{234}\text{U}] \lambda_{\text{Th}} = (2.75 \text{ d.p.m.l}^{-1}) (9.19 \times 10^{-6} \text{ yr}^{-1}) = 2.52 \times 10^{-5} \text{ d.p.m.l}^{-1} \text{ yr}^{-1}$, $\beta_{\text{Pa}} = [^{235}\text{U}] \lambda_{\text{Pa}} = (0.108 \text{ d.p.m.l}^{-1}) (2.17 \times 10^{-5} \text{ yr}^{-1}) = 2.33 \times 10^{-6} \text{ d.p.m.l}^{-1} \text{ yr}^{-1}$, where λ_{Th} and λ_{Pa} are the decay constants for ^{230}Th and ^{231}Pa . ^{231}Pa and ^{230}Th are thus produced at a constant activity ratio of 0.093.

Both nuclides are particle-reactive and are rapidly scavenged to the sea floor. In surficial sediments, however, they are found in ratios varying widely from the constant ratio of their production rates. In the Pacific, sediments deposited in central gyre regions, where particle flux to the sea floor is low, have $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0 < 0.093$ (where $\text{ex.}^{231}\text{Pa}_0$ and $\text{ex.}^{230}\text{Th}_0$ are the activities of ^{231}Pa and ^{230}Th not supported by U present in the sediment mineral lattices, decay-corrected to the time of deposition), but sediments deposited in the more productive ocean margins and upwelling regions have $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0 > 0.093$ (refs 20–22). This basin-wide fractionation during the removal of ^{231}Pa and ^{230}Th from the water column is a result of differential partitioning of the two nuclides between a vertical flux due to uptake by settling particles and a lateral flux due to intensified scavenging in high-particle-flux regions^{21,23,24}. The latter process is referred to as “boundary scavenging”^{25,26}. Thorium is extremely particle-reactive and has a very short residence time (τ_{Th}) in the water column:

FIG. 2 *a*, $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ ratio (see text) in Holocene sediments^{29,33,43,45–49}. Because of the absence of significant systematic regional variability, we use the arithmetic mean for all samples north of 50°S (0.060 ± 0.04 ; 2 s.e.; $n = 68$) as a first approximation to the mean ratio ($[\text{Pa}/\text{Th}]_{\text{Al}}$) for the entire Atlantic north of 50°S . *b*, $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ ratio in LGM sediments. Because of increased boundary scavenging off Western Africa, the standard error on $[\text{Pa}/\text{Th}]_{\text{Al}}$ north of 45°S was calculated by averaging $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ ratios in the upwelling (0.13) and non-upwelling (0.057 ± 0.007 ; 2 s.e.; $n = 26$) regions, and assuming that the upwelling region encompassed 3% of the Atlantic surface area (that is $[\text{Pa}/\text{Th}]_{\text{Al}} = [0.97 \times 0.057] + [0.03 \times 0.13] = 0.059$). *c*, $\text{ex.}^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ ratios in the LGM core section relative to that of the Holocene section from the same core ($(\text{Pa}/\text{Th})_{\text{ex}}/(\text{Pa}/\text{Th})_{\text{G/H}}$). $(\text{Pa}/\text{Th})_{\text{ex}}/(\text{Pa}/\text{Th})_{\text{G/H}} > 1$ indicates enhanced scavenging of ^{231}Pa during LGM, while $(\text{Pa}/\text{Th})_{\text{ex}}/(\text{Pa}/\text{Th})_{\text{G/H}} < 1$ indicates enhanced removal by horizontal mixing. *d*, Ratio of total preserved flux (rain rate minus dissolution on the sea floor) during LGM to total preserved flux during Holocene ($F_{\text{total}}/F_{\text{G/H}}$). Preserved fluxes (F_{total}) were calculated using the known vertical flux of ^{230}Th ($0.85\beta_{\text{Th}}$) as a reference: $F_{\text{total}} = 0.85\beta_{\text{Th}}z/\text{ex.}^{230}\text{Th}_0$, where z is the water depth at the coring site and $\text{ex.}^{230}\text{Th}_0$ is the activity of excess ^{230}Th decay-corrected to the time of deposition. ^{230}Th was measured by inductively coupled plasma mass spectrometry and ^{231}Pa by α -particle counting. Precision and accuracy of the analyses were assessed by replicate analyses of a well characterized reference material in which all uranium-series isotopes are in secular equilibrium (Harwell uraninite)⁵⁰. (?) denotes points which were not used in the calculation of the mean $(\text{Pa}/\text{Th})_{\text{ex}}/(\text{Pa}/\text{Th})_{\text{G/H}}$ and $(F_{\text{total}})_{\text{G/H}}$.



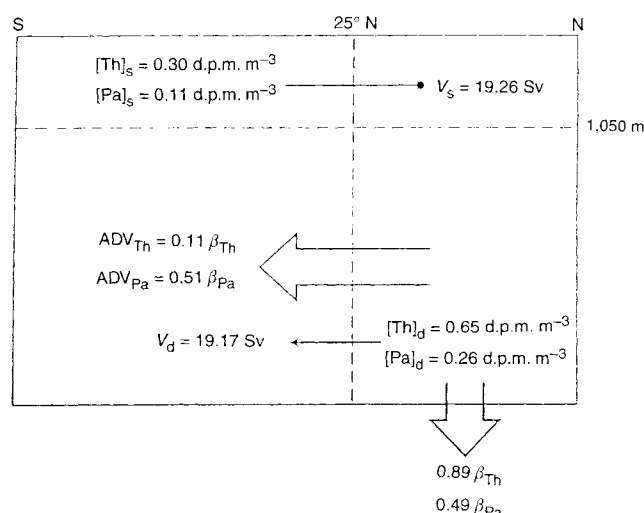


FIG. 3 Horizontal transport of dissolved ^{230}Th and ^{231}Pa in the Atlantic at 25°N from estimates of meridional water transport¹ and water column activity profiles (ref. 51, and M.P.B. and A. P. Fleer, unpublished data). There is a northward meridional flow (V_s) of 19.26 Sv in the upper 1,050 m of the water column, which is balanced by a nearly equivalent southward flow (V_d) below 1,050 m (NADW). The average ^{230}Th concentration above $[\text{Th}]_s$ and below $[\text{Th}]_d$ 1,050 m is 0.30 d.p.m. m^{-3} and 0.65 d.p.m. m^{-3} , respectively. The net southward transport of ^{230}Th across 25°N is thus 6.74×10^5 d.p.m. s^{-1} . The volume of sea water in the Atlantic north of 25°N is estimated at $7.7 \times 10^{15} \text{ m}^3$, resulting in a rate of ^{230}Th production of 61.5×10^6 d.p.m. s^{-1} . The meridional circulation thus causes a net southward transport of 11% of the ^{230}Th , and 89% is scavenged in the underlying sediments. This is also consistent with data from moored sediment traps deployed in the North Atlantic for an entire year (Sargasso Sea, NABE stations), which recorded annual vertical fluxes of ex- ^{230}Th equivalent to $85 \pm 6\%$ (2 standard err.; $n = 6$) of the rate of production from decay of ^{234}U in the overlying water column⁴⁵, suggesting lateral transport of $\sim 15\%$ of the ^{230}Th (mainly advective, as boundary scavenging in the Atlantic is weak). For ^{231}Pa , average activities $[\text{Pa}]_s$, $[\text{Pa}]_d$ are 0.11 d.p.m. m^{-3} ($< 1,050 \text{ m}$) and 0.26 d.p.m. m^{-3} ($> 1,050 \text{ m}$). Hence, the net southward transport is 2.9×10^6 d.p.m. s^{-1} , which amounts to 51% of the ^{231}Pa production rate north of 25°N (5.7×10^6 d.p.m. s^{-1}).

$\tau_{\text{Th}} = 1/\lambda_{\text{Th}} [A_{\text{Th}-230}/A_{\text{U}-234}] = 1.09 \times 10^5 \text{ yr}$ [$0.65 \text{ d.p.m. m}^{-3}/2,750 \text{ d.p.m. m}^{-3}$] = 26 yr, where $A_{\text{Th}-230}$ and $A_{\text{U}-234}$ are the mean seawater activities of ^{230}Th and ^{234}U . Consequently, very little ^{230}Th can be transported laterally before scavenging, and the removal of ^{230}Th is dominated by the vertical flux on settling particles. In comparison, ^{231}Pa is less particle-reactive and has a longer residence time (τ_{Pa}) in the water column: $\tau_{\text{Pa}} = 4.61 \times 10^4 \text{ yr}$ [$0.26 \text{ d.p.m. m}^{-3}/108 \text{ d.p.m. m}^{-3}$] = 111 yr. It can thus be transported laterally over longer distances, to be preferentially scavenged in areas of high particle flux.

Although clearly apparent in Pacific sediments^{20–22}, the effect of boundary scavenging in the Atlantic is only weakly expressed, as ex- $^{231}\text{Pa}_0/\text{ex-}^{230}\text{Th}_0$ ratios in Holocene sediments barely reach the production rate ratio in the upwelling region off Western Africa and do not exhibit particularly high values at the other margin areas (Fig. 2a). The salient features in the Atlantic data are the invariably low ex- $^{231}\text{Pa}_0/\text{ex-}^{230}\text{Th}_0$ (< 0.093) north of 50°S and the high ex- $^{231}\text{Pa}_0/\text{ex-}^{230}\text{Th}_0$ (> 0.093) south of 50°S . We believe that the low ratios and the lack of expression of boundary scavenging in the Atlantic are both the result of the short mean residence time of deep water in this ocean basin (~ 100 years²⁷ or ~ 275 years²⁸), a time that is equivalent to the residence time of ^{231}Pa in the water column of the Atlantic and the timescale for basin-wide lateral mixing²¹ (~ 100 years for a basin 6,000 km wide, and a lateral eddy diffusion coefficient of $3 \times 10^7 \text{ cm}^2 \text{ s}^{-1}$). As deep water (and dissolved elements) are exported from the Atlantic on

a timescale similar to that of scavenging and horizontal mixing, both advection and scavenging are equally important in removing ^{231}Pa from Atlantic water. As a result, boundary scavenging cannot be fully expressed, and much of the ^{231}Pa produced in the Atlantic is exported to the south before being removed in the underlying sediments²⁹.

The fraction of ^{231}Pa exported from the modern Atlantic Ocean as a result of the thermohaline circulation (ADV_{Pa}) can be calculated from the mean ex- $^{231}\text{Pa}_0/\text{ex-}^{230}\text{Th}_0$ in Holocene Atlantic sediments north of 50°S ($[\text{Pa}/\text{Th}]_{\text{Atl}}$): $[\text{Pa}/\text{Th}]_{\text{Atl}} = 0.060 \pm 0.004$ (Fig. 2a). Because the residence time of ^{230}Th in the water column is significantly shorter than the mean residence time of deep water in the Atlantic, 80%–90% of the ^{230}Th produced in the Atlantic is scavenged before it can be exported by advection (Fig. 3). If we assume that 15% of the ^{230}Th is exported ($\text{ADV}_{\text{Th}} = 0.15$), the proportion of ^{231}Pa advected from the modern Atlantic is:

$$\begin{aligned} \text{ADV}_{\text{Pa}} &= 1 - [([\text{Pa}/\text{Th}]_{\text{Atl}} \times (1 - \text{ADV}_{\text{Th}})/0.093)] \\ &= 1 - [(0.060 \times 0.85)/0.093] \\ &= 0.45 \end{aligned} \quad (1)$$

that is, about 45% of the ^{231}Pa produced in the Atlantic water column is advected into the Southern Ocean. This estimate is consistent with the net southward transport of ^{231}Pa based on the distribution of ^{231}Pa activities in the water column and the meridional flows across 25°N (Fig. 3).

Export rates of Atlantic waters

Calculation of NCW residence time. Because ^{231}Pa activities in the deep Atlantic and Southern Ocean are similar³⁰, Southern Component Water (SCW; Fig. 1) minimally affects the ^{231}Pa mass balance in the Atlantic Ocean, and $[\text{Pa}/\text{Th}]_{\text{Atl}}$ reflects only the average residence time of NCW in the Atlantic ($\tau_{\text{NCW,Atl}}$). If we consider a simple one-box model, representing NADW or GNAIW at steady state, where the rate of removal of ^{231}Pa by scavenging ($[\text{Pa}]_{\text{sw}}/\tau_{\text{scav,Pa}}$) and advection ($[\text{Pa}]_{\text{sw}}/\tau_{\text{NCW,Atl}}$) equals its rate of production (β_{Pa}), then:

$$\beta_{\text{Pa}} = ([\text{Pa}]_{\text{sw}}/\tau_{\text{scav,Pa}}) + ([\text{Pa}]_{\text{sw}}/\tau_{\text{NCW,Atl}}) \quad (2)$$

where $[\text{Pa}]_{\text{sw}}$ is the mean ^{231}Pa activity in NADW or GNAIW, and $\tau_{\text{scav,Pa}}$ is the residence time of ^{231}Pa in the water column with respect to scavenging. Recognizing that

$$([\text{Pa}]_{\text{sw}}/\tau_{\text{NCW,Atl}}) = \beta_{\text{Pa}} \text{ADV}_{\text{Pa}} \quad (3)$$

and combining (2) and (3), we obtain

$$\tau_{\text{NCW,Atl}} = \frac{[\beta_{\text{Pa}} - (\beta_{\text{Pa}} \text{ADV}_{\text{Pa}})] \tau_{\text{scav,Pa}}}{\beta_{\text{Pa}} \text{ADV}_{\text{Pa}}} \quad (4)$$

Although based on a very simple model, equation (4) describes reasonably well the mean deep-water ventilation rate in the modern Atlantic. The residence time of ^{231}Pa in the water column of the modern Atlantic is about 110 years, with about 50% removal by scavenging. Hence, $\tau_{\text{scav,Pa}}$ and $\tau_{\text{NCW,Atl}}$ are both approximately 220 years. The latter is consistent with the ^{14}C age of deep waters in the Atlantic²⁸.

Glacial versus modern NCW residence time. If there had been a total shutdown of the conveyor belt circulation during the LGM, with GNAIW overlying deep water of southern origin and forming a closed loop within the Atlantic at intermediate depth (Fig. 1b, a), we would expect the sedimentary ex- $^{231}\text{Pa}_0/\text{ex-}^{230}\text{Th}_0$ in the glacial Atlantic to resemble that of the modern Pacific^{20,24}, with a clear expression of the effect of boundary scavenging at the margins off West Africa and the Equator, and a significantly higher $[\text{Pa}/\text{Th}]_{\text{Atl}}$ approaching the production rate ratio. Although there is indication of a more pronounced boundary scavenging in the equatorial Atlantic during the LGM (Fig. 2b, c), $[\text{Pa}/\text{Th}]_{\text{Atl}}$ in

the LGM sections of Atlantic sediments north of 45°S (0.059 ± 0.007 ; Fig. 2b) is statistically indistinguishable from the Holocene mean (0.060 ± 0.004 ; Fig. 2a). Although our data coverage does not allow us to preclude the possibility of a regional ^{231}Pa sink within the glacial Atlantic, the ex. $^{231}\text{Pa}_0/\text{ex. } ^{230}\text{Th}_0$ of this region would have to be impossibly high to account for a significant fraction of the ^{231}Pa deficit observed in Fig. 2b. We thus conclude that this deficit implies export of NCW from the Atlantic during the LGM and argues strongly against the deep water circulation scenario depicted in Fig. 1a.

Increased boundary scavenging (Fig. 2c) could reflect either a longer $\tau_{\text{NCW,Atl}}$ (that is, a smaller NCW advection rate) or a higher contrast in scavenging intensity between the equatorial region and the other Atlantic regions. Before applying equation (4) to calculate $\tau_{\text{NCW,Atl}}$ during the LGM, however, we must constrain the rate of advection of ^{230}Th (ADV_{Th}) and the rate of scavenging of ^{231}Pa ($\tau_{\text{scav,Pa}}$) in the glacial Atlantic.

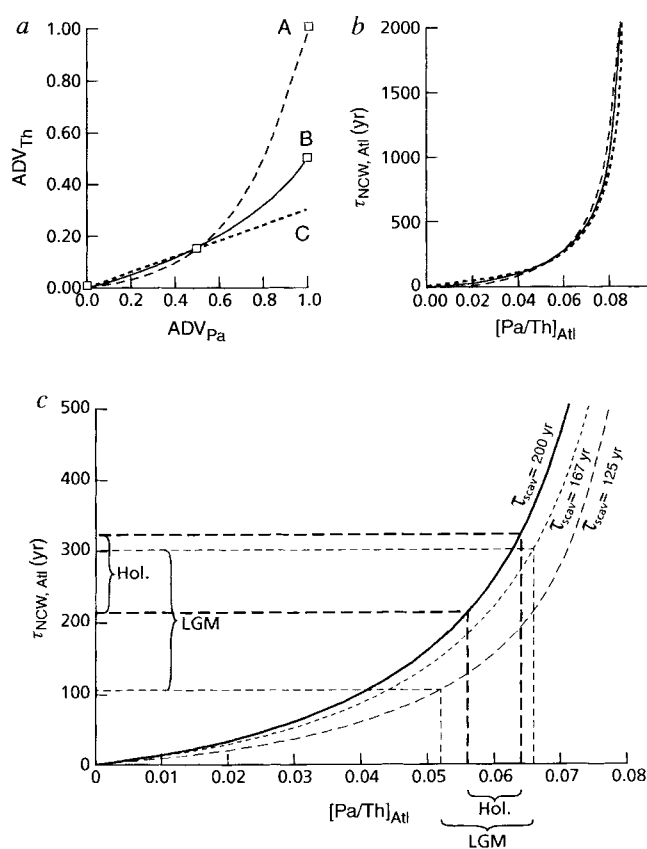


FIG. 4 a, Evaluation of the fraction of ^{230}Th advected from the Atlantic by NCW (ADV_{Th}) as a function of ADV_{Pa} . In the modern ocean, $\text{ADV}_{\text{Pa}} = 0.5$ and $\text{ADV}_{\text{Th}} = 0.15$. If NCW formation ceases totally, $\text{ADV}_{\text{Th}} = \text{ADV}_{\text{Pa}} = 0$. If NCW formation is so rapid as to remove advectively all the ^{231}Pa ($\text{ADV}_{\text{Pa}} = 1$), all the ^{230}Th could also be removed advectively ($\text{ADV}_{\text{Th}} = 1$). However, because ^{230}Th is very particle-reactive, it is conceivable that its advective transport could be much less. To test the sensitivity of this unknown on the calculation of $\tau_{\text{NCW,Atl}}$, we also consider $\text{ADV}_{\text{Th}} = 0.5$ when $\text{ADV}_{\text{Pa}} = 1$, and fit the points with two exponential functions: $\text{ADV}_{\text{Th}} = (0.03213 \exp(3.47\text{ADV}_{\text{Pa}})) - 0.03213$ (function A); $\text{ADV}_{\text{Th}} = (0.0.1131 \exp(1.69\text{ADV}_{\text{Pa}})) - 0.1131$ (function B). As an extreme case, we also assume that ADV_{Th} varies linearly with ADV_{Pa} : $\text{ADV}_{\text{Th}} = 0.3\text{ADV}_{\text{Pa}}$. b, ADV_{Pa} is calculated iteratively by combining functions A, B and C and equation (1), and the residence time of NCW in the Atlantic ($\tau_{\text{NCW,Atl}}$) is calculated as a function of the mean ex. $^{231}\text{Pa}_0/\text{ex. } ^{230}\text{Th}_0$ in Atlantic sediments ($[\text{Pa}/\text{Th}]_{\text{Atl}}$) using equation (4) (lines on figure correspond to a). c, Influence of mean scavenging intensity in the Atlantic on $\tau_{\text{NCW,Atl}}$ estimates and evaluation of $\tau_{\text{NCW,Atl}}$ during the Holocene ($\tau_{\text{scav,Pa}} = 220 \text{ yr}$; $0.053 < [\text{Pa}/\text{Th}]_{\text{Atl}} < 0.063$) and the LGM ($180 \text{ yr} > \tau_{\text{scav,Pa}} > 120 \text{ yr}$; $0.056 < [\text{Pa}/\text{Th}]_{\text{Atl}} < 0.068$).

Although $\text{ADV}_{\text{Th}} \ll \text{ADV}_{\text{Pa}}$, it is not entirely negligible and probably varies with $\tau_{\text{NCW,Atl}}$. As the variation of ADV_{Th} with $\tau_{\text{NCW,Atl}}$ is unknown, we used three predictive equations to evaluate ADV_{Th} from ADV_{Pa} (Fig. 4a). Notwithstanding the relatively large uncertainty regarding the extent of ^{230}Th advection when export fluxes of NCW are high, the associated error in $\tau_{\text{NCW,Atl}}$ is relatively small (Fig. 4b).

On the other hand, because ADV_{Pa} essentially constrains $\tau_{\text{NCW,Atl}}/\tau_{\text{scav,Pa}}$, $\tau_{\text{NCW,Atl}}$ reflected by a given $[\text{Pa}/\text{Th}]_{\text{Atl}}$ decreases as the mean scavenging intensity increases (Fig. 4c), and the error in the estimated $\tau_{\text{NCW,Atl}}$ will be identical to that for $\tau_{\text{scav,Pa}}$. Because scavenging rate is controlled primarily by particle flux through the water column and, to some extent, by the chemical composition of settling particles^{31,32}, we can attempt to constrain $\tau_{\text{scav,Pa}}$ during the LGM from changes in the flux and nature of the particles reaching the sea floor. To this end, we have estimated the preserved particle flux to the sea floor (that is, vertical rain rate minus dissolution on the sea floor), using the known vertical flux of ex. ^{230}Th as a reference^{33–35}, and calculated the ratios of LGM to Holocene flux (Fig. 2d).

The preserved particle flux was 1.4 ± 0.2 (2 standard error; $n = 27$) times larger during the LGM, primarily because of increased flux of terrigenous particles^{34,35}. Because of the shoaling of the Atlantic lysocline during the LGM³⁶, the contrast in particle rain might have been more pronounced than suggested by the preserved flux ratio. There are also reports of increased diatom productivity in the equatorial Atlantic during the LGM^{37–39}, which could account for the higher LGM ex. $^{231}\text{Pa}_0/\text{ex. } ^{230}\text{Th}_0$ found in this region (Fig. 2b, c). These observations thus strongly suggest that the mean scavenging rate in the Atlantic increased during the LGM. In an attempt to bracket the mean residence time of ^{231}Pa in the glacial Atlantic with respect to scavenging ($(\tau_{\text{scav,Pa}})^{\text{LGM}}$), we have assumed that relative scavenging intensities are inversely related to relative preserved particle fluxes 1.4 ± 0.2 (5). This probably tends to underestimate the increase in scavenging rate in the LGM Atlantic, as it does not take into account increased carbonate dissolution which would cause the flux contrast to be underestimated, or increased opal production, which may enhance ^{231}Pa scavenging³²: $(\tau_{\text{scav,Pa}})^{\text{Hol}} = [\tau_{\text{Pa}}/(1 - \text{ADV}_{\text{Pa}})]^{\text{Hol}} = 111 \text{ yr}/0.55 = 200 \text{ yr}$, $(\tau_{\text{scav,Pa}})^{\text{Hol}}/1.6 < (\tau_{\text{scav,Pa}})^{\text{LGM}} < (\tau_{\text{scav,Pa}})^{\text{Hol}}/1.2$, $125 \text{ yr} < (\tau_{\text{scav,Pa}})^{\text{LGM}} < 167 \text{ yr}$.

We can thus bracket the mean residence time of NCW in the Atlantic ($\tau_{\text{NCW,Atl}}$; Fig. 4c).

For the Holocene ($\tau_{\text{scav,Pa}} = 200 \text{ yr}$; $0.056 < [\text{Pa}/\text{Th}]_{\text{Atl}} < 0.064$): $213 \text{ yr} < \tau_{\text{NCW,Atl}} < 323 \text{ yr}$. For the LGM ($125 \text{ yr} < \tau_{\text{scav,Pa}} < 167 \text{ yr}$; $0.052 < [\text{Pa}/\text{Th}]_{\text{Atl}} < 0.066$): $105 \text{ yr} < \tau_{\text{NCW,Atl}} < 301 \text{ yr}$.

The results suggest no change within the uncertainty of the estimates ($\pm 150 \text{ yr}$), but probably a small reduction in $\tau_{\text{NCW,Atl}}$ during LGM (that is, a small increase in NCW export rate), because $(\tau_{\text{scav,Pa}})^{\text{LGM}}$ might have been underestimated. The increased boundary scavenging in the glacial Atlantic (Fig. 2c) must thus reflect a higher contrast in scavenging intensity between the equatorial region and the other Atlantic regions^{37–39} rather than a significant increase in NCW residence time. Improving on these estimates will require a more extensive sampling coverage and a more detailed scavenging model.

The Atlantic benthic $\delta^{13}\text{C}$ and Cd/Ca records require formation of shallower GNAIW during the LGM^{5–9}. The new information provided by $[\text{Pa}/\text{Th}]_{\text{Atl}}$ is that its rate of production and export from the glacial Atlantic were similar to those of the modern NADW. Therefore, we conclude that the smaller contrast in nutrient concentrations between the deep Atlantic and the deep Pacific during the LGM⁴⁰ was caused not by a slowdown of the thermohaline circulation but by the invasion of the deep Atlantic by water of southern origin. Nutrient proxies and Pa/Th provide complementary information that is required to understand changes in deep-water circulation. While palaeonutrient proxies are better for showing the vertical restructuring of the conveyor belt, $[\text{Pa}/\text{Th}]_{\text{Atl}}$ is better for quantifying variations in water exchange between ocean basins.

Sensitivity of $[\text{Pa}/\text{Th}]_{\text{Atl}}$ tracer. In order to be a useful palaeo-circulation proxy, $[\text{Pa}/\text{Th}]_{\text{Atl}}$ must respond rapidly to changes in NCW residence time. For a hypothetical instantaneous shut-down of the NADW, the response time of $[\text{Pa}/\text{Th}]_{\text{Atl}}$ would be similar to the residence time of ^{231}Pa in the water column (about 110 years). As ^{230}Th and ^{231}Pa removal by advection from the Atlantic is stopped, the concentrations of both radionuclides and the rates of their removal by scavenging would gradually increase toward a new steady state: $[\text{Pa}]_t = ([\text{Pa}]_0 - (\beta_{\text{Pa}}/k_{\text{Pa}}))e^{-(k_{\text{Pa}}t)} + \beta_{\text{Pa}}/k_{\text{Pa}}$, $[\text{Th}]_t = ([\text{Th}]_0 - (\beta_{\text{Th}}/k_{\text{Th}}))e^{-(k_{\text{Th}}t)} + \beta_{\text{Th}}/k_{\text{Th}}$, $([\text{Pa}/\text{Th}]_{\text{Atl}})_t = ([\text{Pa}]_t/[\text{Th}]_t) = (([\text{Pa}]_0 - (\beta_{\text{Pa}}/k_{\text{Pa}}))e^{-(k_{\text{Pa}}t)} + \beta_{\text{Pa}}/k_{\text{Pa}})/(([\text{Th}]_0 - (\beta_{\text{Th}}/k_{\text{Th}}))e^{-(k_{\text{Th}}t)} + \beta_{\text{Th}}/k_{\text{Th}})$, where $[\text{Pa}]_0$ and $[\text{Th}]_0$ are the average activities of ^{231}Pa and ^{230}Th in the deep Atlantic before the shut-down of NADW, and k_{Pa} and k_{Th} are the scavenging rate constants (that is, $1/\tau_{\text{scav}}$).

$[\text{Pa}/\text{Th}]_{\text{Atl}}$ should thus approach 0.093 within 500 years of a hypothetical cessation of NCW export (Fig. 5). This is sufficiently rapid that possible changes in $\tau_{\text{NCW,Atl}}$ associated with abrupt events such as the Younger Dryas⁴¹ or the Heinrich events⁴² could be recorded in the ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ ratios of high-sedimentation-rate cores.

Further constraints on ocean circulation

Although the evidence based on $[\text{Pa}/\text{Th}]_{\text{Atl}}$ precludes a significant slowdown of NCW export from the Atlantic Ocean during the LGM, it does not clarify whether CPDW was an isolated water mass accumulating nutrients, as argued from the Southern Ocean benthic $\delta^{13}\text{C}$ record¹⁵, or whether it was an integral part of the glacial conveyor belt circulation, as suggested by the Cd/Ca record^{10,16}. We can resolve this question by calculating the mean sedimentary ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ in the southern ocean ($[\text{Pa}/\text{Th}]_{\text{soc}}$). If CPDW was isolated from the glacial conveyor belt, $[\text{Pa}/\text{Th}]_{\text{soc}}$ should approach the production rate ratio (0.093) during the LGM.

Although sample coverage for the southern ocean is still sparse, a distinct pattern in the distribution of sedimentary ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ is beginning to emerge (Fig. 6a). In Holocene sediments, comparatively high ratios (0.23 ± 0.03 , 2 standard error, $n = 4$) occur at the Polar Frontal Zone in the Atlantic and western Indian sector, associated with high opal flux. Significantly lower ratios occur in the subantarctic region of these sectors and throughout the eastern Indian sector (0.13 ± 0.01 , 2 standard error, $n = 14$). This distribution pattern suggests that ^{231}Pa advected from the Atlantic is rapidly scavenged as it enters the CPDW in the South Atlantic, consistent with the high affinity of Pa for opal³². To the north, the ratios drop below 0.093 (0.06 ± 0.01 , 2 standard error, $n = 19$). Similarly low ratios appear to prevail near the Antarctic continent (0.06 ± 0.04 , 2 standard error, $n = 3$).

Little in this pattern seems to have changed during the LGM,

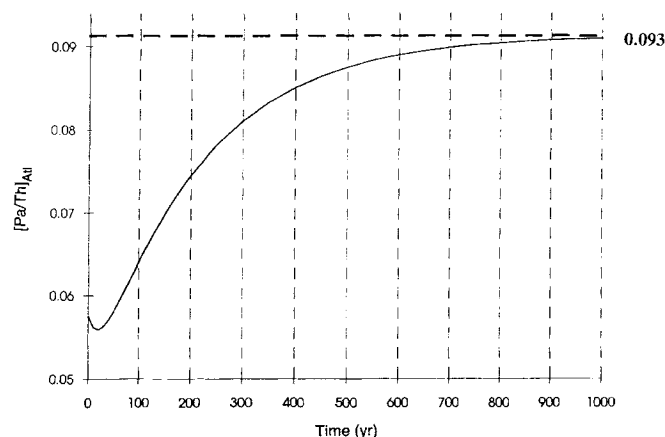


FIG. 5 Response time of the average ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ in Atlantic sediments ($[\text{Pa}/\text{Th}]_{\text{Atl}}$) to a total and instantaneous shutdown of NCW export from the Atlantic Ocean.

except for a northward shift in the zone of high ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ in the Atlantic sector (Fig. 6b), which corresponds to a similar change in the position of the PFZ^{43,44}. The mean values for all the ratios > 0.093 and for each time period are statistically indistinguishable (Holocene: $[\text{Pa}/\text{Th}]_{\text{soc}} = 0.16 \pm 0.02$, $n = 18$; LGM: $[\text{Pa}/\text{Th}]_{\text{soc}} = 0.16 \pm 0.02$, $n = 15$), indicating that as much ^{231}Pa reached CPDW during the LGM as today. This observation is further strong evidence against the isolation of CPDW during the LGM (Fig. 1B, b) and for the interpretation based on Cd/Ca that CPDW was part of the glacial conveyor belt, with nutrient concentration intermediate between the deep Pacific and the deep Atlantic (Fig. 1B, c).

If we assume that $[\text{Pa}/\text{Th}]_{\text{soc}}$ calculated for the Atlantic and Indian sector is valid for the entire Southern Ocean, the ^{231}Pa mass balance for the Southern Ocean can be calculated. The depositional flux of ^{231}Pa during the Holocene and LGM southern ocean sediments ($[(\beta_{\text{Th}}V_{\text{soc}}) + (0.15\beta_{\text{Th}}V_{\text{Atl}})]$) $[\text{Pa}/\text{Th}]_{\text{soc}} = [(2.52 \times 10^{-2} \text{ d.p.m. m}^{-3} \text{ yr}^{-1} \times 123 \times 10^{15} \text{ m}^3) + (3.76 \times 10^{-3} \text{ d.p.m. m}^{-3} \text{ yr}^{-1} \times 263 \times 10^{15} \text{ m}^3)]/0.165 = 6.7 \times 10^{14} \text{ d.p.m. yr}^{-1}$ essentially balances *in situ* production ($\beta_{\text{Pa}}V_{\text{soc}} = 2.33 \times 10^{-3} \text{ d.p.m. m}^{-3} \text{ yr}^{-1} \times 123 \times 10^{15} \text{ m}^3 = 2.9 \times 10^{14} \text{ d.p.m. yr}^{-1}$) and ^{231}Pa import from the Atlantic ($0.5\beta_{\text{Pa}}V_{\text{Atl}} = 0.5 \times 2.33 \times 10^{-3} \text{ d.p.m. m}^{-3} \text{ yr}^{-1} \times 263 \times 10^{15} \text{ m}^3 = 3.1 \times 10^{14} \text{ d.p.m. yr}^{-1}$) where V_{soc} and V_{Atl} are the total volume of seawater in the Southern Ocean and the Atlantic, respectively. The latter is equivalent to the rate of ^{231}Pa production in the entire Southern Ocean and is therefore an important consideration for the interpretation of past changes in the ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ of Southern Ocean sediments. This mass balance calculation also suggests relatively little net exchange of ^{231}Pa between the Pacific and the Southern Ocean. Although we might expect to find a small export to the Southern Ocean by eddy diffusion due to boundary scavenging, there is no extensive advective transport as observed in the Atlantic.

Conclusion

Sedimentary $^{231}\text{Pa}/^{230}\text{Th}$ is a valid proxy for changes in deep-water circulation during the past 150 kyr. It complements nutrient

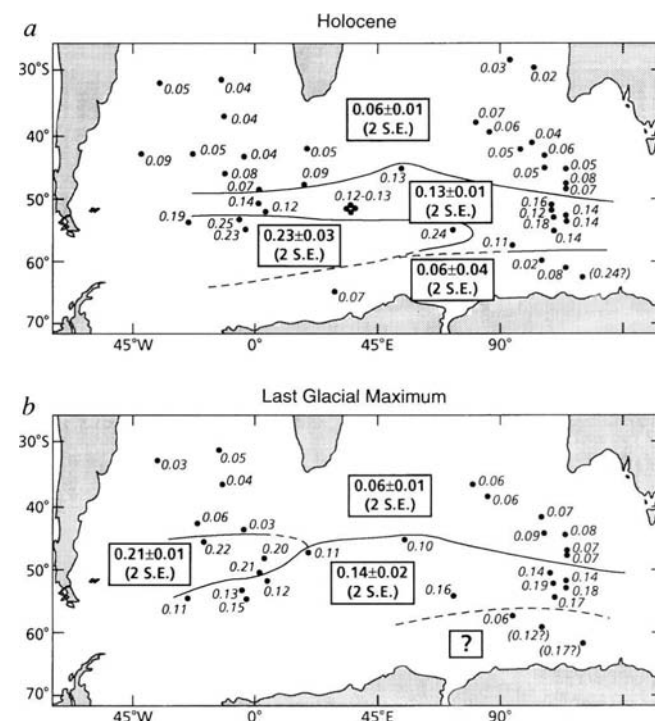


FIG. 6 Distribution of ex. $^{231}\text{Pa}_0/\text{ex.}^{230}\text{Th}_0$ in Southern Ocean sediments^{29,33,43,45,52,53}. a, Holocene; b, LGM.

proxies by adding important constraints on advective fluxes of deep water between ocean basins. Because advection is as important as scavenging for the removal of ^{231}Pa from Atlantic deep water, the effect of uncertainties resulting from past changes in scavenging rates is mitigated.

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Atlantic $^{231}\text{Pa}/^{230}\text{Th}$ ratios indicate that GNAIW export during the LGM was at least as intensive as NADW export in the modern ocean, and southern ocean $^{231}\text{Pa}/^{230}\text{Th}$ ratios indicate that CPDW was—as it is now—an integral part of the glacial conveyor belt (Fig. 1c). □

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RNA recognition and translational regulation by a homeodomain protein

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In *Drosophila*, the primary determinant of anterior pattern is the gradient morphogen bicoid (bcd), a homeodomain protein that binds DNA and transcriptionally activates target genes at different threshold concentrations. Here we present evidence that bcd also binds RNA and acts as a translational repressor to generate an opposing gradient of the homeodomain protein caudal (cad). RNA binding by bcd seems to involve direct interactions between the bcd homeodomain and discrete target sequences within the 3' untranslated region of the cad messenger RNA and to block the initiation of cad translation.

In *Drosophila*, the anterior pattern of the embryo is controlled primarily by the graded expression of bicoid (bcd), a homeodomain-containing protein^{1,3}. During oogenesis, bcd mRNA is tightly localized to the anterior pole of the egg^{4,5}. After fertilization, this mRNA is translated giving rise to an anterior-to-posterior gradient of bcd protein which then activates the transcription of target genes at distinct concentration thresholds^{2,3,6,7}. Transcriptional activation by bcd is mediated by direct binding of the homeodomain to DNA targets⁸.

Shortly after the bcd gradient forms, a second homeodomain protein, caudal (cad)⁹, accumulates in an opposing posterior-to-anterior gradient under bcd control^{2,10,11}. The regulation of cad by bcd seems to be necessary for normal patterning, as inappropriate

anterior expression of cad causes deletions of head and thoracic segmentation^{12,13}. However, several lines of evidence indicate that this regulation occurs post-transcriptionally^{10,11}. Most strikingly, bcd controls the formation of a cad gradient in laid but unfertilized eggs (Fig. 1), in which all genes are thought to be transcriptionally silent.

Here we show that the regulation of cad by bcd occurs at the level of translation, and depends on both the bcd homeodomain and on *cis*-acting sequences in the 3' untranslated region (UTR) of the cad message. We also show that the bcd homeodomain can bind specifically to these *cis*-acting sequences *in vitro*. Finally, we provide evidence that bcd regulates cad expression by blocking translational initiation.

bcd response element in *cad* 3' UTR

To map regions of the *cad* mRNA or protein required for the regulation of *cad* by *bcd*, we constructed a chimaeric transgene designed to express maternal *cad-lacZ* transcripts resembling the endogenous *cad* transcript, but coding for a *cad*- β gal fusion protein (Fig. 2a, transcript i). Embryos derived from females carrying this transgene express a gradient of *cad*- β gal protein that is similar to that of endogenous *cad*; moreover, formation of this *cad*- β gal gradient is dependent on *bcd* (data not shown). Removal of the *cad* 5' UTR from this transcript does not alter its ability to be regulated by *bcd* (Fig. 2a, transcript iii, and b, lower). However, replacement of the *cad* 3' UTR with the 3' UTR of another gene, the *Adh2* gene from *Drosophila mulleri*¹⁴, leads to loss of regulation (Fig. 2a, transcript ii, and b, upper). Hence sequences in the 3' UTR, but not the 5' UTR, of *cad* mRNA are necessary for its regulation by *bcd*. These results also argue against the possibility that *bcd* downregulates *cad* by destabilizing *cad* protein.

To define the minimal RNA sequences necessary for the regulation of *cad* by *bcd*, we generated a series of chimaeric transgenes that express maternal transcripts composed of the *cad* 5' UTR, the coding sequence of the *fushi tarazu* (*ftz*) gene, part or all of the first 940 nucleotides of the 3' UTR of *cad*, and the 3' UTR of the *Drosophila mulleri* *Adh2* gene (Fig. 2c). Maternal *ftz*-encoding transcripts which contain a discrete 342-nucleotide segment of the *cad* 3' UTR are translated to form a *ftz* gradient (Fig. 2c, and d, upper), whereas transcripts lacking part or all of this region give rise to uniform *ftz* expression (Fig. 2c, and d, lower). Moreover, the ability of this segment of the *cad* 3' UTR to mediate downregulation of *ftz* expression is dependent on *bcd* (Fig. 2d, middle). Hence, we refer to this portion of the *cad* 3' UTR as the *bcd* response element (BRE).

cad regulation requires *bcd* homeodomain

To determine which part of the *bcd* protein is required for *cad* regulation, we first examined the ability of forms of *bcd* protein truncated at either the amino or the carboxy terminus to repress *cad* expression *in vivo*. The results of this analysis (summarized in Fig. 3a) indicate that a central 159-amino-acid region of *bcd* containing the 61-amino-acid homeodomain is necessary to regulate *cad* expression. We then examined the activity of the *bcd*^{E6} mutant protein, which contains an in-frame deletion of 11 amino acids within the homeodomain⁷, and found that it is unable to regulate *cad* even though the mutant protein is expressed like the wild-type protein *in vivo* (data not shown). Finally, we assayed the activities of a series of *bcd* mutant proteins which have amino acid substitutions in the recognition helix (helix III) of the homeodomain (Fig. 3b, c)^{15,16}. Substitutions at positions 1, 2 and 5 of the recognition helix, all of which retain normal DNA binding specificity^{15,16}, also retain the ability to regulate *cad* expression (data not shown). In contrast, substitution of the lysine normally found at

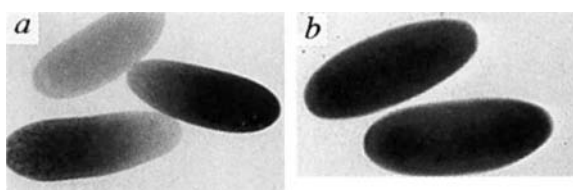


FIG. 1 Regulation of *bcd* by *cad* occurs post-transcriptionally. Expression of *cad* in laid but unfertilized eggs derived from wild-type (a) or *bcd*^{E1} (b) females. Shortly after eggs are laid, *cad* protein begins to accumulate differentially in the posterior half of eggs derived from wild-type females (a; protein accumulation has not yet begun in one of the three eggs shown), but uniformly in eggs derived from *bcd* mutant females (b). Laid but unfertilized eggs complete meiosis, but all four haploid nuclei remain at the egg surface as polar bodies and are believed to be transcriptionally inactive.

METHODS: The *bcd*^{E1} allele and *cad* staining are described in Figs 2 and 3.

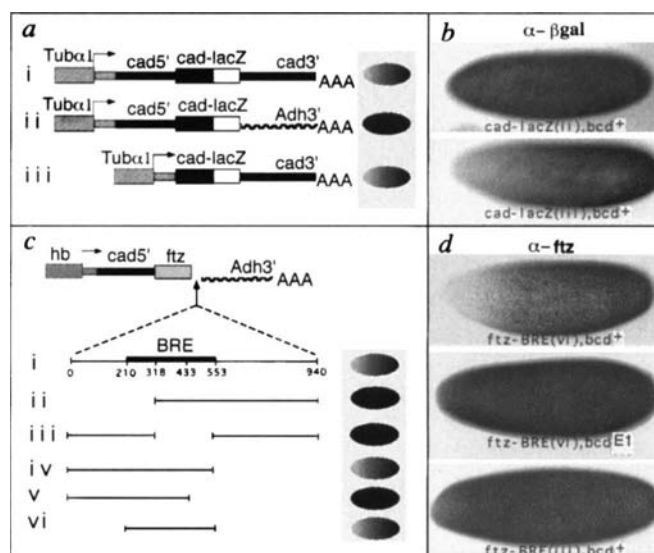


FIG. 2 The *cad* 3' UTR contains a *cis*-acting *bcd* response element (BRE) required to mediate translational repression by *bcd*. a, *Tubx1-cad-lacZ* gene (i) and derivatives (ii, iii) used to determine whether *cis*-acting sequences required for post-transcriptional repression of *cad* map to the 5' UTR, the coding sequence, or the 3' UTR. Downregulation of *cad*- β gal expression in the anterior half (as shown on the right) depends on sequences which map to the 3' UTR. b, Expression of *cad*- β gal is uniform in embryos obtained from females carrying *Tubx1-cad-lacZ* transgene (ii), which lacks the *cad* 3' UTR, but is downregulated by *bcd* in embryos from females carrying transgene iii, which has the *cad* 3' UTR intact but lacks the *cad* 5' UTR. c, The *hb-ftz-cad* 3' UTR gene (i) and derivatives (ii-vi) used to map the BRE in the *cad* 3' UTR. Downregulation of *ftz* expression in the anterior half (shown right) depends on a 342-nucleotide segment of the *cad* 3' UTR, the BRE. d, Expression of *ftz* obtained from maternal transcripts of *hb-ftz-cad* transgene vi, which contains the BRE, is regulated in embryos obtained from *bcd*⁺ females, but uniform in embryos obtained from *bcd*^{E1} mutant females. Expression of *ftz* obtained from the *hb-ftz-cad* 3' UTR transgene iii, which lacks the BRE, is uniform irrespective of *bcd* gene function.

METHODS. The *Tubx1-cad-lacZ* gene (ai) is composed of a 2.6-kb segment of DNA bearing the constitutive promoter of the *Tubulin* α 1 gene^{31,32} positioned upstream of the *cad* 5' UTR, a chimaeric *cad-lacZ* coding sequence, and the *cad* 3' UTR. The *cad* 5' UTR sequence begins at position 615 (ref. 9), and extends 219 nucleotides to the ATG. The *cad-lacZ* coding sequence contains the entire *lacZ* coding sequence (including the *lacZ* stop codon) inserted in frame at position 2,071, which is 41 nucleotides upstream from the *cad* stop codon. The *cad* 3' UTR sequence extends from the stop codon (position 2,112) to position 3,007, which is 44 nucleotides downstream from the polyadenylation site. The two derivatives (a ii and a iii) of the *Tubx1-cad-lacZ* transgene (ai) were generated by, respectively, replacing the *cad* 3' UTR sequences with a DNA fragment containing the 3' UTR and polyadenylation signal of the *Adh2* gene from *Drosophila mulleri* (nucleotides 3,553–3,803)¹⁴, or by removing the *cad* 5' UTR sequences. Note that the *Tubx1* DNA encodes the first 175 nucleotides of the mature *Tubx1* 5' UTR, which precedes the *cad* 5' UTR in the mature *Tubx1-cad-lacZ* transcript. The *hb-ftz-cad* 3' UTR transgene (ci) is composed of a 4.7-kb segment of DNA bearing both zygotic and maternal promoters from the *hb* gene³³ positioned upstream of a chimaeric gene composed of the *cad* 5' UTR (see above), the *ftz* coding sequence, a 936-bp segment of *cad* DNA, including 40 base pairs immediately upstream of the stop codon and the first 896 nucleotides of the *cad* 3' UTR, and a DNA fragment containing the 3' UTR and polyadenylation sequences from the *D. mulleri* *Adh2* gene (see above). Note that the *hb* DNA encodes the first 162 nucleotides of the maternal *hb* 5' UTR, which precede the *cad* 5' UTR in the mature transcript. The various derivatives of this *hb-ftz-cad* gene (cii–cvi) are identical to the canonical *hb-ftz-cad* 3' UTR gene (ci) except for the deletion of portions of the *cad* 3' UTR generated using existing restriction sites (*Syl*, *Ssp*, *Afl*, *Pst*) at positions 2,281, 2,390, 2,460 and 2,625, respectively (see ref. 13 for details). Expression of *ftz* and β gal was detected by immunohistochemistry as described previously⁷ using rabbit anti-*ftz*³⁴ and anti- β gal (Cappel) antisera.

position 9 (*bcd*^{K9}) with either glutamine (*bcd*^{Q9}) or alanine (*bcd*^{A9}) blocks the regulation of *cad* *in vivo* (Fig. 3c), even though both mutant proteins are expressed normally (Fig. 3c) and retain the ability to activate transcription through altered DNA targets^{15,16}. Taken together, these results implicate the *bcd* homeodomain in the regulation of *cad* expression.

bcd homeodomain binds the BRE

To test the possibility that *bcd* can bind directly to the BRE full-length *bcd* protein was expressed in Cos cells, and extracts from these cells were used in RNA binding assays with radiolabelled BRE RNA. We found that *bcd* can bind the BRE in both north-western and ultraviolet crosslinking assays (Fig. 4). In the north-western assay, proteins were first separated by electrophoresis, electroblotted to nitrocellulose filters, and then incubated with radiolabelled BRE RNA (Fig. 4 legend). Using this assay, a radiolabelled band (Fig. 4a, lane 1) of similar electrophoretic mobility to that of *bcd* (Fig. 4b, lane 4) is detected in extracts from Cos cells expressing intact *bcd*, but not in extracts from cells expressing *bcd*^{E6} protein or from untransfected Cos cells (Fig. 4a, lanes 2 and 3). Moreover, the protein in this complex can be immunoprecipitated with *bcd*-specific antibodies (Fig. 4a, lanes 4–6, 4b, lanes 4–6), indicating that it is *bcd*. In the ultraviolet crosslinking assay (Fig. 4c), Cos cell lysates were irradiated with ultraviolet light in the presence of radiolabelled BRE RNA, and the resulting RNA–protein complexes were separated by gel electrophoresis. We again detected a radiolabelled complex of similar size to *bcd* (Fig. 4a, b) in extracts from Cos cells which express *bcd*, but not in extracts from *bcd*^{E6}-expressing or mock-transfected Cos cells (Fig. 4c; and data not shown). Formation of this complex can be completely competed by the addition of a 40-fold excess of unlabelled BRE RNA, but is only slightly

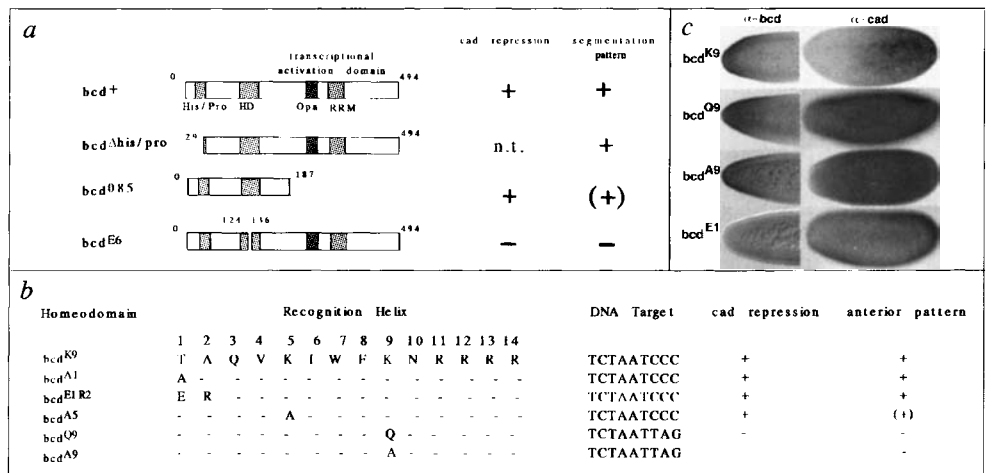
competed by the addition of 200-fold excess of unlabelled RNA from the *Tubulin α 1* (*Tub α 1*) 3' UTR (Fig. 4c). Taken together, these data indicate that *bcd* can bind preferentially to the BRE, and that deletion of 11 amino acids in the *bcd*^{E6} homeodomain abolishes this binding.

Because both genetic and biochemical evidence indicate that the *bcd* homeodomain is required for *cad* regulation (Figs 3 and 4), we tested the ability of the homeodomain on its own to bind radiolabelled BRE RNA *in vitro*. The homeodomain peptide was produced in *Escherichia coli*, and binding experiments were performed using total cell lysates. The *bcd* homeodomain can bind the BRE both in ultraviolet crosslinking (Fig. 5a) and gel-shift (Fig. 5b–d) assays.

In the crosslinking experiment we detected two prominent radiolabelled complexes of relative molecular mass approximately 15,000 and 17,000 (*M_r* 15K and 17K) in extracts from cells expressing the *bcd* homeodomain (Fig. 5a, lane 2). When the same nitrocellulose membrane was then probed with polyclonal anti-*bcd* antibodies, two prominent bands of similar electrophoretic mobility were detected (Fig. 5a, lane 1) indicating that a *bcd* homeodomain peptide is present in both complexes. Radiolabelled complexes are barely detectable in a similar experiment using extracts from cells expressing homeodomains derived from either the *Ultrabithorax* (*Ubx*) gene of *Drosophila*, or the *goosoid* (*gsc*) gene of *Xenopus* (Fig. 5a, lanes 3 and 4).

For the gel-shift assay, homeodomain containing lysates were incubated with radiolabelled RNA, and analysed by native polyacrylamide gel electrophoresis (PAGE) (Fig. 5b–d). Binding of the *bcd* homeodomain to the BRE was almost completely competed by the addition of a 50-fold excess of unlabelled BRE RNA, and was undetectable in the presence of a 250-fold excess of unlabelled BRE. In contrast, the addition of a 250-fold excess of

FIG. 3 Post-transcriptional regulation of *cad* requires the *bcd* homeodomain *in vivo*. a, The amber mutation *bcd*⁰⁸⁵ truncates the protein after amino acid 187, and removes several distinct C-terminal domains including a proposed RNA recognition motif (RRM³⁵), the OPA repeat, as well as most of the serine- and threonine-rich sequences required for transcriptional activation. Nevertheless, embryos derived from *bcd*⁰⁸⁵ mutant females show reduced accumulation of *cad* anteriorly, indicating that the mutant protein retains the ability to regulate *cad* expression (data not shown; similar results were obtained for the *bcd*¹¹¹ and *bcd*^{E5} mutant proteins which truncate the protein after amino acids 262 and 264, respectively). The *bcd*^{ΔHis-Pro} mutation, which lacks the first 29 amino acids of *bcd*, including most of the previously defined His–Pro repeat motif, does not appear to interfere with normal *cad* regulation because a transgene encoding a *bcd*^{ΔHis-Pro} mutant protein lacking these amino acids can completely rescue absence of endogenous *bcd* gene function (P. M. Macdonald, unpublished observations). Conversely, the *bcd*^{E6} mutation, which encodes a protein lacking 11 amino acids within the homeodomain⁷, fails to regulate *cad* expression (see text). b, The wild-type allele (*bcd*^{K9}) and the various mutant alleles were introduced into the *Drosophila* genome in the context of an 8-kb 'rescuing' fragment of genomic DNA from the *bcd* locus⁷. The resulting *bcd*^{K9}, *bcd*^{Δ1}, *bcd*^{E1} and *bcd*^{E1/R2} transgenes all behave like wild-type *bcd* alleles: a single copy of each transgene can rescue the mutant phenotype associated with the *bcd*^{E1} mutation and allows formation of a *cad* gradient. The *bcd*^{Δ5} transgene also rescues the ability to regulate *cad*, but does not fully restore normal head segmentation, possibly because the mutant protein may be less stable¹⁵. In contrast, the *bcd*^{Q9} and *bcd*^{A9} transgenes show no evidence of *bcd* gene function. Unaltered amino acids are represented by dashes and the DNA target specificity shown is that determined previously^{15,16} in yeast and flies. c, Expression of *bcd* and *cad* in embryos derived from *bcd*^{K9} (wild type), *bcd*^{Q9}, *bcd*^{A9} and *bcd*^{E1} females.



The *bcd*^{Q9} and *bcd*^{A9} proteins form anterior-to-posterior gradients which appear indistinguishable from that of *bcd*^{K9} protein (all embryos are oriented with anterior to the left; *bcd* staining is shown in optical cross-section). Unlike embryos derived from *bcd*^{K9} females, in which *cad* forms an opposing posterior-to-anterior gradient, *cad* accumulates uniformly in embryos derived from *bcd*^{Q9}, *bcd*^{A9} and *bcd*^{E1} females (*cad* staining is shown in surface views). The *bcd*^{E1} mutation is an apparent null allele which results in an unstable protein with no observable biological activity². METHODS. A 378-bp PstI–BspMII segment from the *bcd* coding sequence (positions 1193–1571)⁴ containing each of the mutations was introduced in place of the corresponding wild-type sequence in an 8-kb fragment of *bcd* genomic DNA, and the resulting transgenes were inserted into the *Drosophila* genome as described previously^{7,36}. The construct encoding the His–Pro deletion is identical to the wild-type *bcd* genomic rescuing fragment, except that the first 29 codons have been deleted. Three independent insertions of the *bcd*^{Q9} and *bcd*^{A9} transgenes were tested for activity. Expression of *bcd* and *cad* was assayed in embryos before nuclear cycle 13 detected by immunohistochemistry using rabbit anti-*bcd*⁷ and anti-*cad*¹⁰ antisera.

unlabelled *Tubx1* 3' RNA did not compete binding (Fig. 5b, compare lanes 3 and 4 with 5 and 6). Gel-shift experiments were also performed with radiolabelled RNA from the *Tubx1* 3' UTR, the *Adh2* 3' UTR, and the *bcd* 3' UTR. Binding by the *bcd* homeodomain to these RNA probes was not detected (Fig. 5c). Together these results indicate that the *bcd* homeodomain can bind specifically to the BRE.

To further define the RNA binding site for the *bcd* homeodomain, we generated three non-overlapping RNAs which together span the length of the BRE. As shown in Fig. 5d, the *bcd* homeodomain binds two of these transcripts (A and B) but is unable to bind the third (C). Although transcripts A and B share only limited sequence homology (see Fig. 5, legend) they appear to be bound by the *bcd* homeodomain with similar affinity (data not shown; ref 13). Thus the BRE contains at least two distinct *bcd* binding sites in the BRE, one in region A and one in region B, but there may be more. It is also possible that additional binding sites are present elsewhere in the *cad* 3' UTR.

Translational repression of *cad* by *bcd*

To examine how *cis*-acting elements in the 3' UTR of *cad* mediate regulation of translation by *bcd*, we constructed the hybrid genes shown in Fig. 6. The gene in Fig. 6a encodes a dicistronic transcript with an internal ribosome entry site (IRES) inserted between the first and second cistron (*cad-lacZ* and *ftz*, respectively) and the *cad* 3' UTR (including the BRE) at the 3' end. The gene in Fig. 6c is identical to that in Fig. 6a, except that the first cistron is now *ftz* and the second cistron is *cad-lacZ*. It has been shown previously that this IRES, derived from the *Antennapedia* (*Anp*) leader, mediates internal ribosome entry and translational initiation in a cap-independent manner¹⁷. Hence translation of the first cistron (*cad-lacZ* in Fig. 6a and *ftz* in Fig. 6c) should be cap-dependent, whereas translation of the second cistron (*ftz* in Fig. 6a and *cad-lacZ* in Fig. 6c) should be cap-independent. Both dicistronic transcripts were expressed during oogenesis under control of the *hb* promoter (Fig. 6 legend), and protein derived from each cistron was assayed in early embryos by immunohistochemistry. As shown in Fig. 6b, d, translation of the 5' cistron of each dicistronic transcript is repressed by *bcd*, whereas translation of the downstream IRES-associated cistron is not. Hence we infer that *bcd* bound to the 3' UTR of *cad* mRNA blocks translational initiation at the 5' end, possibly by interfering with a step that depends on the 5' cap structure.

Discussion

Homeodomain proteins define a major class of eukaryotic transcription factors which bind DNA in a site-specific manner and activate or repress transcription, depending on other functional domains in the protein. Here we present evidence that the homeodomain-containing protein *bcd* also functions as an RNA binding translational repressor. Specifically, we have shown: (1) that *bcd* downregulates *cad* protein expression by a post-transcriptional mechanism; (2) that this regulatory mechanism depends *in vivo* on both the *bcd* homeodomain and a discrete *cis*-acting regulatory sequence (the *bcd* response element, or BRE) in the 3' UTR of the *cad* mRNA; (3) that the *bcd* homeodomain can bind specifically to the BRE *in vitro*; and (4) that the BRE fails to mediate repression by *bcd* when translation initiates at an internal ribosomal entry site rather than at the 5' end of the transcript. Thus we postulate that *bcd* binds the 3' UTR of *cad* mRNA by means of interaction between the homeodomain and the BRE, and then blocks a 5' cap-dependent step of translational initiation.

How might *bcd* bound to the 3' UTR of *cad* mRNA block translation initiated at the 5' end? *cis*-acting elements required for translation regulation have been identified in the 3' UTRs of several mRNAs (reviewed in ref. 18); however, the regulatory mechanisms involved are poorly understood. In the case of *cad*, our data define a critical *trans*-acting factor (*bcd*) that binds to the *cis*-acting response element (the BRE) in the *cad* 3' UTR and suggest that *bcd* bound to the BRE blocks a 5' cap-dependent step

of translational initiation.

At least one other protein, poly(A) binding protein, seems to bind at the 3' end of transcripts and, like *bcd*, to influence cap-dependent initiation at the 5' end⁹⁻¹¹. Hence, interactions that normally occur between the 3' and 5' ends of the message, such as those involving poly(A) binding protein, might serve to bring the BRE to the vicinity of the initiating machinery, and thereby enable *bcd* and perhaps other factors associated with BRE to block

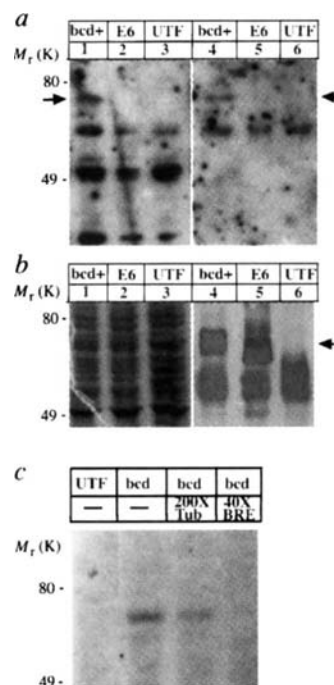


FIG. 4 Binding of *bcd* to target sequences in the BRE. **a**, Northwestern blot of lysates from Cos cells expressing *bcd* protein using radiolabelled BRE RNA. Proteins were separated by SDS-PAGE, electroblotted to nitrocellulose membrane, and then incubated with radiolabelled BRE RNA. Lanes 1 and 2 contain lysates from cells expressing intact *bcd*⁺ and *bcd*^{E6} mutant protein, respectively. Lane 3 contains lysate from untransfected Cos cells (UTF). Lanes 4–6 show a similar northwestern blot after immunoprecipitation with anti-*bcd* polyclonal antibodies. Arrows indicate the position of the complex of *bcd* and BRE RNA. **b**, Coomassie-stained gel of Cos cell lysates (lanes 1–3) and western blot immunodetection after immunoprecipitation with the same *bcd*-specific antiserum (lanes 4–6; note that the heavy chain of the *bcd* antibody is also detected as a band of lower *M_r* in these three lanes). Although the *bcd*^{E6} protein is present at similar levels to the intact *bcd* protein, and is recognized by the *bcd* antibody (compare lanes 4 and 5, arrow), it does not appear to bind to the BRE (Fig. 4a, lanes 2 and 5). **c**, Ultraviolet crosslinking of radiolabelled BRE RNA and lysates from Cos cells expressing full-length *bcd* protein or mock-transfected controls (UTF). Lysates were irradiated with ultraviolet in the presence of radiolabelled BRE RNA. Excess probe was then degraded with RNAase A and the products analysed by SDS-PAGE (see methods, Fig. 5 legend). A radiolabelled complex similar in size to that observed in the northwestern and western blot experiments shown in **a** and **b** forms in *bcd*-expressing extracts, but not in extracts from mock-transfected Cos-cells. The formation of this complex is completely competed by the addition of a 40-fold excess of unlabelled BRE RNA, but only slightly competed by a 200-fold excess of unlabelled *Tubx1* 3' UTR RNA, indicating that it is sequence specific. **METHODS.** Cos7 cells were transfected by the DEAE-dextran method³⁷ using expression vectors in which either the wild-type *bcd* complementary cDNA, or a *bcd* cDNA containing the E6 deletion, were fused downstream of the major adenovirus promoter³⁸. Control cells were mock-transfected with buffer¹³ without DNA and treated in parallel with *bcd*-producing cells. Extracts and both the northwestern and crosslinking assays were as performed previously^{13,39}. All binding reactions were carried out in the presence of 100 µg ml⁻¹ transfer RNA. Immunoprecipitations were performed with immunoprecipitin (Gibco BRL), according to the manufacturer's protocol. Preparation of RNA probes and ultraviolet crosslinking experiments are described in the legend of Fig. 5.

translation. Another possibility is that bcd bound to the BRE directly antagonizes the ability of poly(A) binding protein to interact with initiation factors at the 5' cap and so prevents translation. One mechanism that appears unlikely is that bcd mediates cleavage and subsequent degradation of the cad transcript. Such a mechanism is not consistent with previous observations that the endogenous *cad* mRNA remains uniformly distributed, even when the protein gradient is established^{10,11}, and would also be expected to destabilize and hence down-regulate, translation of both cistrons instead of just the 5' cistron in the dicistronic transcripts shown in Fig. 6.

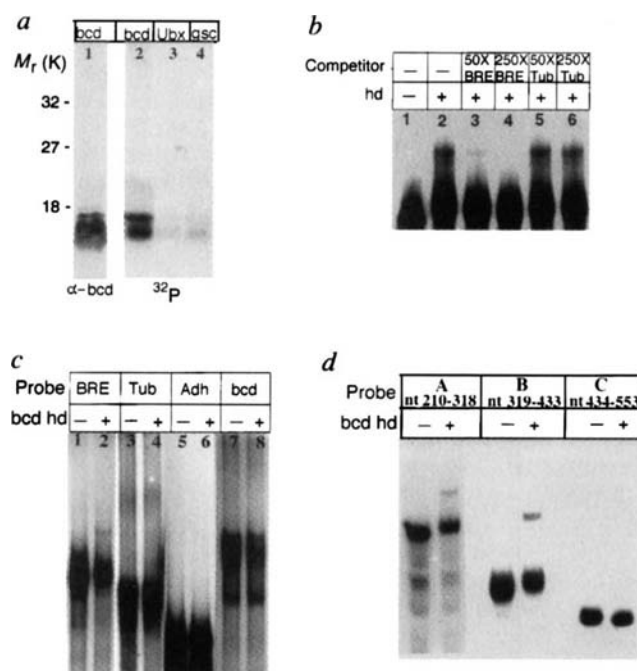
How does the bcd homeodomain recognize both RNA and DNA targets in a sequence-specific manner? One obvious difference between recognition of RNA and DNA is that DNA targets are recognized mainly as double-stranded helices, whereas RNA targets have the additional potential to adopt more complex tertiary conformations. In addition, the helical structure of double-stranded RNA is significantly different from that of double-stranded DNA. In particular, in a perfectly paired RNA helix, the major groove is too narrow and deep for amino-acid side chains to gain access to base functional groups. Conversely, the minor groove, which is both wide and shallow enough for such contact to occur, provides fewer hydrogen-bond acceptors and donors to facilitate specific interactions with amino-acid side chains²².

FIG. 5 Binding of the bcd homeodomain to at least two target sequences in the BRE. **a**, Lysates from bacterial cells expressing either the bcd, Ubx or gsc homeodomains were crosslinked with radiolabelled BRE RNA, treated with RNase, separated by denaturing PAGE, and then electroblotted to nitrocellulose membrane. The bcd homeodomain is able to bind the BRE RNA, resulting in formation of a pair of radiolabelled complexes of apparent M_r ~15K and 17K, respectively (lane 2). Complexes of similar electrophoretic mobility are detected when the same nitrocellulose filter is probed with polyclonal anti-bcd antibodies (lane 1). Note that lane 2 detects only bcd homeodomain that is crosslinked to the radiolabelled BRE, whereas lane 1 detects both crosslinked and free bcd homeodomain. Binding of gsc and Ubx homeodomains to the BRE is barely detectable with this assay (lanes 3 and 4). All three homeodomains were present at equal levels, as determined by Coomassie blue staining (not shown). For the gel-shift assay, lysates from bacterial cells expressing the bcd homeodomain were incubated with radiolabelled BRE RNA (**b**), and analysed by native PAGE. Competitor RNA used was either unlabelled BRE RNA or unlabelled *Tubx1* 3' UTR RNA, and was added in the amounts indicated above each lane. As shown, binding of the bcd homeodomain to the BRE can be competed by the addition of unlabelled BRE RNA but not by the addition of unlabelled *Tubx1* 3' UTR (compare lanes 3 and 4 with 5 and 6). **c**, As in **b**, bacterial cell lysates either lacking (–) or containing equal amounts (+) of homeodomain were incubated with radiolabelled probes, and binding was analysed by a gel-shift assay. In this case the probes used were: the BRE (lanes 1 and 2), the *Tubx1* 3' UTR (lanes 3 and 4), and *Adh2* 3' UTR (lanes 5 and 6), or the *bcd* 3' UTR (lanes 7 and 8). Note that the BRE RNA is bound by the bcd homeodomain-containing lysate, whereas the *Tub*, *Adh2* and *bcd* 3' UTRs are not (**d**). The BRE was divided into three non-overlapping fragments (A, B and C; nucleotide numbers as indicated), and radiolabelled transcripts were generated from each region. Gel-shift analysis was performed as in **c**. Transcripts A and B but not C are shifted by the bcd homeodomain. Although isolated C RNA is not bound *in vitro*, this region is required *in vivo* for translational repression (Fig. 2). In principle, fragment C might contain a bcd binding site which functions in the intact BRE but fails to bind bcd because it cannot fold properly in isolation. Alternatively, it might contain the binding site for another factor necessary to allow bcd bound to sites in fragments A and B to repress translation. Two short sequence repeats, GTTTCGAC and GTTAAT, are present in both A and B, but not C.

METHODS. The *bcd*⁴⁹, *Ubx* and *gsc* homeodomain peptides were each expressed in bacteria using a pET-14b vector (Novagen) to drive expression of a 63-amino-acid peptide including the 60-amino-acid homeodomain as well as three upstream amino acids (Met, Gly and Arg). RNA probes were generated by *in vitro* transcription using either T3 or T7 RNA polymerase. BRE RNA was generated from nucleotides 210 to 553 from the *cad* 3' UTR. Transcripts A, B and C were generated by subcloning nucleotides 210–318, 319–433 and 434–553, respectively, downstream from the T3 RNA polymerase promoter in Bluescript. Fragment A includes 35 nucleotides from the Bluescript polylinker (*KpnI*–*EcoRV*) not present in transcripts B or

Despite these structural constraints, there are several examples of protein recognition in the major groove of double-stranded RNA^{23–28}. In these cases, recognition appears to occur in the vicinity of bulges and loops, which can distort the helical structure making the major groove accessible²⁹. The HIV proteins Tat and Rev, for example, seem to recognize RNA targets by means of interactions between highly basic and arginine-rich protein domains and the major groove of RNA stem-loop structures in the vicinity of bulged nucleotides^{26,28}. For Rev, this basic domain has been shown to bind its target as an α -helix³⁰.

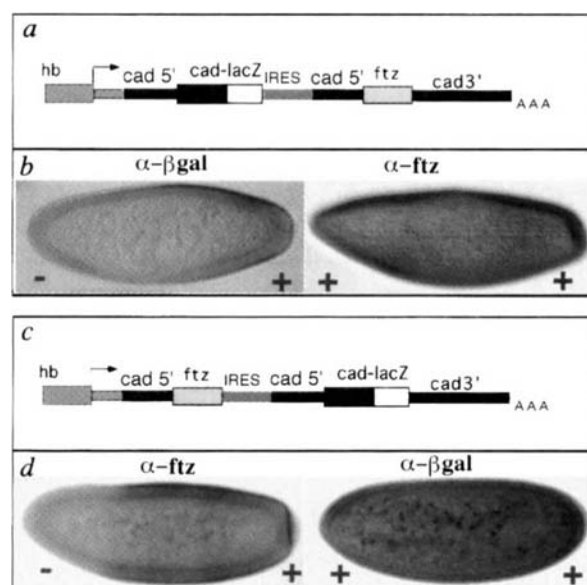
The properties of the RNA binding domain of Rev are of interest because this domain has at least two features in common with the recognition helix of the bcd homeodomain. First, as is the case for all known homeodomain proteins, the bcd recognition helix is basic and thought to be α -helical. Second, the C-terminal portion of the bcd recognition helix is rich in arginine (See Fig. 3b). Hence one possibility is that the bcd recognition helix contacts RNA in a similar manner to the basic domain of Rev. Other portions of the bcd homeodomain, such as the N-terminal arm, are also rich in arginine, and might stabilize this interaction by making additional contacts with the RNA. The possibility that the recognition helix, initially defined by its role in DNA binding, may also be involved in interactions with RNA is supported by our observation that substitutions of amino acid 9 in the bcd recognition helix can block translational repression of *cad* (Fig. 3).



C. The *D. mulleri* *Adh2* 3' UTR transcript was generated from a fragment extending from the stop codon to an *SspI* site 250 nucleotides downstream of the polyadenylation signal¹⁴. The *Tubx1* 3' UTR probe was generated from a fragment extending from the *Scal* site near the stop codon to a *SphI* site approximately 480 nucleotides downstream⁴⁰. The bcd 3' UTR probe was generated from a fragment extending from the *EcoRV* site close to the stop codon to the *FspI* 480 nucleotides downstream. Radiolabelled RNA probes were gel purified before use, and equal counts were added per sample. In all cases, 1 μ l of bacterial or Cos cell extracts were preincubated with 5 μ g tRNA (except for D, in which 10 μ g tRNA was used) and competitor RNA as indicated for 10 min at room temperature in a total volume of 20 μ l. RNA probes were then added (500,000 c.p.m. for crosslinking assays or 50,000 c.p.m. for gel-shift assays) and incubation was continued for 10 min at room temperature. Gel-shift analysis was as described previously⁴¹. Ultraviolet crosslinking experiments were as described previously³⁹ except that binding reactions were done as described above¹³. Gels were either western blotted and the blots autoradiographed, or fixed, dried and autoradiographed directly.

FIG. 6 Regulation of *cad* by *bcd* at the level of translational initiation. *a*, *c*, Transgenes designed to express maternal dicistronic transcripts in which the first and second cistrons are separated by an internal ribosomal entry site (IRES) and the second cistron is followed by the *cad* 3' UTR (including the BRE). The two transgenes differ only in the order of the cistrons: *cad-lacZ* precedes *ftz* in the transcript in *a*, whereas *ftz* precedes *cad-lacZ* in the transcript in *c*. Note that both cistrons have the same 219-nucleotide *cad* 5' leader sequence upstream of their initiator codons. Translation of the 5' cistron is expected to occur by a cap-dependent mechanism, whereas translation of the 3' cistron is expected to occur through the IRES and to be independent of the cap. *b*, *d*, Optical cross-sections of cycle-14 embryos showing *cad-βgal* and *ftz* expression derived from maternal transcripts of the transgenes shown in *a* and *c*, as indicated. At this stage, most nuclei are located at the surface of the embryo, and can be seen in cross-section around the periphery. For both dicistronic transcripts, protein derived from the first cistron is absent (–) at the anterior pole and present (+) at the posterior pole, whereas protein derived from the second cistron is present (+) at both poles.

METHODS. The dicistronic transgene shown in *a* consists of three components: the *hb-ftz* segment present in the *hb-ftz-cad* 3' UTR transgene describes in Fig. 2*c* (including the *cad* 5' UTR sequences between the *hb* promoter and *ftz* coding sequences); a 250-nucleotide fragment derived from exon 4 of the *Antennapedia* P2 leader¹⁷ (positions 14–265 of cDNA 303/311)⁴², which contains the IRES sequence; and the *cad-lacZ* segment present in the *Tubx1-cad-lacZ* gene described in Fig. 2*a* (including the intact *cad* 3' UTR and associated polyadenylation site). The dicistronic transgene shown in *b* is identical to that in *a* except that the *cad-lacZ* and *ftz* coding sequences have been exchanged. The *hb* promoter drives transcription during oogenesis and in the anterior half of the embryo during early embryogenesis (under *bcd* control). For both transgenes, approximately 50% of embryos derived from females heterozygous for the transgene show evidence of downregulation of the first cistron in the anterior half of the body between cycles 10 and 12, while the remaining 50% show uniform expression. We infer that the first class is composed of those embryos which did not inherit the transgene, and hence express *ftz* and *cad-βgal* derived solely from maternal transcripts. By cycle 14, the level of anterior



expression derived from zygotic transcription of each transgene greatly exceeds that derived from maternal transcripts, allowing embryos in which protein derives solely from maternal transcripts to be unambiguously distinguished from their siblings. Note that *ftz* derived from the endogenous *ftz* gene is also expressed in a broad central portion of the body during the latter half of cycle 14. This endogenous expression provides an internal control for timing and antibody staining, but does not interfere with analysing *ftz* expression derived from either of the two dicistronic transcripts at the poles. Expression of *ftz* and *βgal* was detected as described in Fig. 2.

However, neither of these substitutions results in a dramatic reduction in RNA binding *in vitro* (not shown; ref. 13). It is possible that the *in vitro* assays used are not discriminating enough to detect subtle binding defects that have pronounced consequences on *cad* regulation *in vivo*. Alternatively, these substitutions might not interfere with RNA binding, but rather block the translational repression of *cad* because they alter the activity of *bcd* once bound.

Our finding that the *bcd* homeodomain can bind specifically to *cad* mRNA poses the question of how general is this type of

interaction. It is possible that the case of *bcd* is atypical, involving an unusual structural aspect of the *bcd* homeodomain that has been co-opted for RNA binding and the translational repression of *cad*. Accordingly, RNA binding might be unique to *bcd*, or occur only among a small subset of related homeodomain proteins. Alternatively, the interaction between *bcd* and BRE may reflect a conserved aspect of homeodomain structure and function, and hence reveal a more general role for this structural motif in recognizing RNA sequences and mediating translational regulation. □

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Early aqueous activity on primitive meteorite parent bodies

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THE interstellar material from which the Solar System formed has been modified by many processes¹: evaporation and condensation in the solar nebula, accretion into protoplanetary bodies and post-accretion processes within these bodies. Meteorites provide a record of these events and their chronology². Carbonaceous CI chondrites are among the most primitive, undifferentiated meteorites^{3–6}, but nevertheless show evidence of post-accretion alteration⁷; they contain carbonates that are believed to have formed by reactions between anhydrous CI precursor materials and circulating fluids in the meteorite parent body (or bodies), yet little is known about the nature of these reactions or the timescale on which they occurred. Here we report measurements of excess ⁵³Cr—formed by the decay of short-lived ⁵³Mn—in five carbonate fragments from the CI chondrites Orgueil and Ivuna. Our results show that aqueous alteration on small protoplanetary bodies must have begun less than 20 Myr after the time of formation of the oldest known solar-nebula condensates² (Allende refractory inclusions). This upper limit is much shorter than that of 50 Myr inferred from previous studies⁸, and clearly establishes aqueous alteration as one of the earliest processes in the chemical evolution of the Solar System.

There are only a few isotope systems that allow determination of the time sequence of early Solar System events². One dating method is based on the evolution of the initial ⁸⁷Sr/⁸⁶Sr ratio. An alternative approach is the use of short-lived isotopes, such as ²⁶Al (ref. 9) and ⁵³Mn (refs 10, 11), radioisotopes that are now extinct but were present in the early Solar System as shown by excesses of the daughter isotopes ²⁶Mg and ⁵³Cr, respectively. In the case of ⁵³Mn, the ⁵³Mn/⁵⁵Mn ratios inferred from ⁵³Cr excesses can be used to determine relative timescales for the formation of the solids in which the Mn–Cr system is measured. Previous Sr-isotope studies

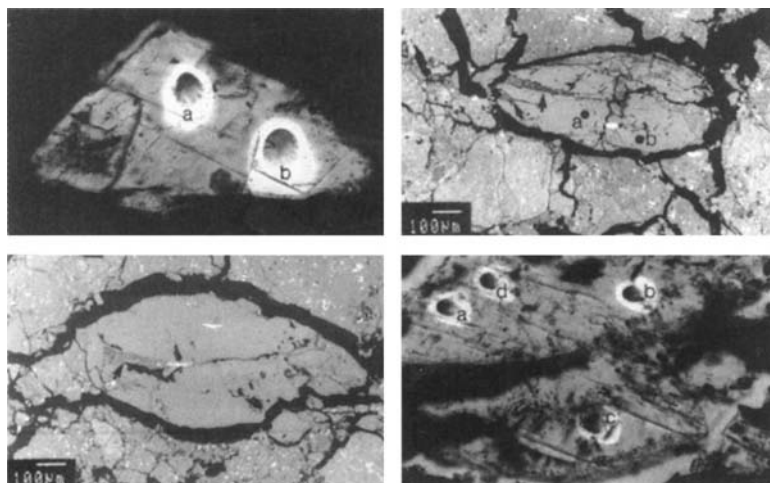
have shown that aqueous alteration on the CI parent body occurred early, and have set an upper limit of ~50 Myr between parent body accretion and the formation of carbonates⁸. Application of the short-lived ⁵³Mn chronometer offers the opportunity to obtain age information on an even finer relative timescale. Here we report isotopic analyses of the Mn–Cr system on five carbonate fragments from the CI chondrites Ivuna and Orgueil.

Carbonates are, next to magnetites, the most abundant coarse-grained mineral phases in CI chondrites, but the abundances of carbonates vary widely both within and among different CI meteorites^{7,12,13}. On average, they constitute ~5 vol.% of the total meteorites. Among CI carbonates, it is important to distinguish between isolated carbonate grains and carbonate fragments^{7,13}. Only carbonate fragments such as those shown in Fig. 1 are apparently remnants of carbonate veins and were formed during very early stages of aqueous alteration on the CI parent body. These polycrystalline and mostly elongated-shaped fragments, which are usually 60 µm to >1 mm in size, generally occur between individual lithic clasts and are not simply derived from identifiable lithological units within the CI meteorites¹³. They have only been observed in Orgueil and Ivuna, and are uniformly distributed throughout the examined thin sections. In contrast, smaller isolated grains, which are normally heterogeneously dispersed in individual lithic clasts, are related to lithological units and apparently have been formed during a later stage of aqueous alteration¹³.

Four chemically distinct types of carbonates are found in CI chondrites^{7,12,13}: dolomite (CaMg(CO₃)₂), breunnerite (Mg(Fe, Mn)(CO₃)₂), calcite (CaCO₃) and Mg, Ca-bearing siderite (FeCO₃). Dolomite is the dominant phase in CIs, breunnerite is much less abundant, calcite and siderite are extremely rare. Dolomite exhibits a wide range of MnCO₃ contents but a more limited range of FeCO₃ contents¹⁰. The compositional variability of dolomite fragments can be expressed as (Ca_{0.45–0.50}Mg_{0.43–0.49}Mn_{0.02–0.09}Fe_{0.03–0.04})CO₃. Because of the combination of high Mn contents and low Cr contents (generally below the detection limit of the electron microprobe used to determine the chemical compositions of CI carbonates), dolomites are ideally suited for a search for ⁵³Cr excesses due to the decay of extinct ⁵³Mn.

In addition to petrographical and mineralogical evidence pointing to early carbonate formation¹³, two previous isotopic studies have obtained formation ages of CI carbonates. Sr-isotope measurements revealed that dolomites and breunnerites in Orgueil formed within 50 Myr after formation of the Orgueil parent body^{8,14}. Furthermore, relatively large variations of ⁸⁷Sr/⁸⁶Sr ratios observed among different carbonates suggest

FIG. 1 Top left, carbonate fragment Orgueil 5 as observed in transmitted light (width of view, 0.8 mm). The two sputter holes (labelled 'a' and 'b') produced by the primary ion beam during SIMS analysis of this fragment are clearly visible. The beam halo of spot 'b' slightly extends into the surrounding matrix material, demonstrating the main problem encountered during ion-probe isotopic measurements of small crystals. Top right, backscattered electron image of carbonate fragment Orgueil 1 (light grey). This fragment contains a large sulphide grain (bright) and veins of phyllosilicate inclusions (arrows). Black dots (labelled 'a' and 'b') indicate the spots analysed by SIMS. Bottom left, backscattered electron image of carbonate fragment Orgueil 2. Like fragment 1 this elongated-shaped, unusually large, fragment has inclusions of sulphide laths (bright) and phyllosilicates (arrow). Bottom right, carbonate fragment Orgueil 2 in transmitted light (width of view, 0.8 mm), showing the analysed spots ('a', 'b', 'c', 'd') and beam haloes after ion-probe isotopic analysis. The typical rhombohedral cleavage planes of carbonates are quite apparent. The labels used for carbonate fragments and analysis spots are the same as those in Fig. 2.



different formation times for different types of CI carbonates⁸. In addition, Scatena-Wachel *et al.*¹⁵ found a ^{53}Cr excess in a breunnerite grain from Orgueil but did not make any conclusions about the timescale of aqueous activity.

The first attempt at applying the ^{53}Mn chronometer to the problem of dating carbonate fragments in the CI chondrite Ivuna by ion microprobe analysis gave a hint of an even greater age (16.5 ± 1.7 Myr after Allende refractory inclusions) for one carbonate fragment¹⁶, than those previously reported but measurements of other fragments from Ivuna and carbonate fragments from the CI chondrites Orgueil and Alais were frustrated by too-low Mn/Cr ratios. Successful detection of ^{53}Cr excesses due to the decay of short-lived ^{53}Mn in small mineral phases by *in situ* secondary ion mass spectrometric analysis requires samples with rather high Mn/Cr ratios, ideally in excess of 1,000. Although many CI dolomites have such high ratios, the analysis does not succeed if tiny Cr-rich inclusions are present in the analysed carbonate or if the fragment is so small that the primary ion beam cannot be focused entirely onto the carbonate and the beam

halo sputters (isotopically normal) Cr from surrounding Cr-rich matrix material (see Fig. 1).

We have successfully measured four carbonate fragments from Orgueil that were free of Cr-rich inclusions and were large enough so that primary beam overlap onto the matrix was not a problem. Analyses were made with the Washington University ion microprobe. The $^{53}\text{Cr}/^{52}\text{Cr}$ ratios measured in meteoritic carbonates were corrected for instrumental mass fractionation, which was determined by comparing the ratios measured in the meteorite matrix with the terrestrial ratio¹⁰. The mass fractionation of -15‰ per atomic mass unit determined in this way is very similar to mass fractionation values obtained in many other materials including sulphides and stainless steel. Furthermore, the excesses observed in the meteoritic carbonates of this study are much larger than any possible matrix effects.

The data obtained on four Orgueil fragments (numbers 1, 2, 5 and 8) are shown in Fig. 2 together with the previously obtained Ivuna data¹⁶. All samples show significant ^{53}Cr excesses, ranging up to $1,150\text{‰}$. For fragment 5, the carbonate with the highest $^{53}\text{Mn}/^{55}\text{Cr}$ ratio (Fig. 2), ^{53}Cr excesses measured on different spots and in different runs are correlated with this ratio. The data points for this fragment and the fragments Orgueil 8 and Ivuna 2 lie on the same line and yield an inferred $^{53}\text{Mn}/^{55}\text{Mn}$ ratio of $(1.99 \pm 0.16) \times 10^{-6}$; the data points of the other two fragments define a line with slope $(1.42 \pm 0.16) \times 10^{-6}$ (the errors on the slope are 1σ). It is not quite clear whether or not the difference between these two ratios is significant. It corresponds to a time difference of 1.8 Myr. An alternative interpretation is that all five carbonate fragments formed at the same time but that the Orgueil fragments 1 and 2 suffered from partial equilibration of the Mn–Cr system that resulted in data points lying below the slope 1.99×10^{-6} line. Partial isotopic equilibration is frequently observed for the Al–Mg system in Ca-, Al-rich inclusions (CAIs) and indicates that the samples suffered thermal events after their original formation¹⁷. Because Cr and Mn are more mobile than Mg and Al, post-formation equilibration cannot be excluded for the carbonate fragments. The essential finding, however, is that all five fragments analysed in this study have ^{53}Cr excesses consistent with inferred $^{53}\text{Mn}/^{55}\text{Mn}$ ratios between 1.42×10^{-6} and 1.99×10^{-6} at the time of their formation. We note that any isotopic equilibration resulting in a lowering of the inferred $^{53}\text{Mn}/^{55}\text{Mn}$ ratio means that the true formation age can only be older.

There are several ways in which the formation age of the CI carbonates can be compared to other early Solar System time markers. If we assume that ^{53}Mn (half-life $\tau_{1/2} = 3.7$ Myr) was homogeneously distributed in the early Solar System, and that the initial $^{53}\text{Mn}/^{55}\text{Mn}$ ratio in the solar nebula at the time of formation of Allende CAIs is given by the value of $(4.4 \pm 1.0) \times 10^{-5}$ measured by Birck and Allegre¹⁰, we conclude that the CI carbonate fragments of this study solidified between 16.5 and 18.3 Myr after Allende CAIs. If, on the other hand, we take the initial $^{53}\text{Mn}/^{55}\text{Mn}$ ratio of 1.29×10^{-6} found for the angrites¹⁸, whose Pb/Pb ages were measured to be 4.5578 Gyr, the formation of CI carbonate fragments (and, therefore, the very early stage of aqueous alteration on the CI parent body) took place between 4.5601 and 4.5583 Gyr before present. With a reported Pb/Pb age of 4.566 Gyr for Allende CAIs¹⁹, this gives a time interval of 5.9–7.7 Myr between the formation of Allende CAIs and CI carbonate fragments. At present, the discrepancy between these two time calibrations is not resolved, although Mn–Cr data from other differentiated meteorites²⁰ are in conflict with the timescale derived from the angrite age. It also might be that the assumption of ^{53}Mn homogeneity is unwarranted²¹.

But whatever the exact calibration of the ^{53}Mn clock may be, and irrespective of the detailed distribution of the ^{53}Mn in the solar nebula, the presence of live ^{53}Mn at a level of $^{53}\text{Mn}/^{55}\text{Mn} = 2 \times 10^{-6}$ in CI carbonates at the time of their formation indicates that only a few half-lives of this radioisotope (at most, 20 Myr) elapsed between the formation of the earliest

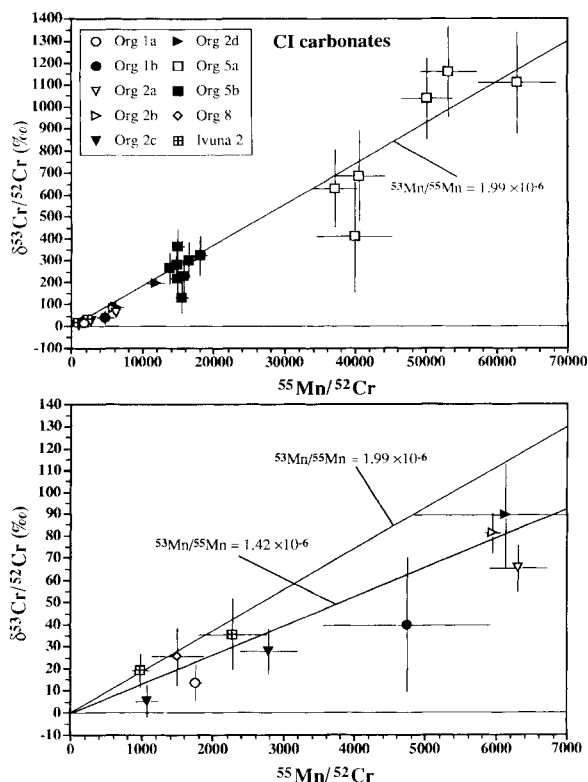


FIG. 2 Top, Mn–Cr evolution diagram for five carbonate fragments from the CI meteorites Orgueil and Ivuna. An expanded-scale view of the lower left-hand corner of this plot is also shown (bottom). Deviation from the terrestrial $^{53}\text{Cr}/^{52}\text{Cr}$ ratio of 0.113458 are plotted as δ values (‰ deviations). The labels a, b and c denote different spots on a given fragment (see also Fig. 1). Several data points for a given spot indicate repeat analyses on that spot. As material is sputtered away during SIMS analysis, the Mn/Cr ratio can change from one analysis to the next. Excesses of ^{53}Cr are observed in all fragments and indicate the presence of ^{53}Mn at the time the carbonates formed on the CI parent body. The line of slope 1.99×10^{-6} is a best-fit line through all data points for Orgueil grain 5 and normal Cr (that is, $\delta^{53}\text{Cr} \equiv 0$ at $^{55}\text{Mn}/^{52}\text{Cr} = 0$). The data points for Orgueil 8 and Ivuna 2 are consistent with this line, the data points for the remaining two fragments fall close to the line of slope 1.42×10^{-6} but, compared to the 1σ analytical errors, the deviations are not large enough to clearly establish that different carbonates formed at different times. In addition, scatter could be caused by disturbance of the Mn–Cr system. For example, the fact that the data points for Orgueil 2 scatter more than the errors from a single line could be due to re-equilibration of the Cr isotopes during or after ^{53}Mn decay.

Solar System objects (CAIs) and pervasive aqueous activity on the parent body of CI chondrites. Aqueous alteration apparently was one of the earliest processes during the evolution of the Solar System. □

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Synthesis of oriented films of mesoporous silica on mica

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Porous inorganic solids find wide application in, for example, catalysis and separation technologies. One of the most promising routes to such materials exploits cooperative interactions between a supramolecular organic template and inorganic precursor molecules to create an ordered porous solid^{1–7}. This general approach offers considerable flexibility, and has led to materials with a wide range of bulk morphologies and controlled pore diameters spanning several orders of magnitude^{8–10}. From a technological point of view, the preparation of such materials in the form of mesoporous thin films is desirable, but attempts to achieve this aim have been largely unsuccessful, resulting in disordered materials¹¹. Here we report the synthesis of thin (~0.2–1.0 µm) ordered films of mesoporous silica on freshly cleaved mica substrates. The films are stable on removal from the substrate. We propose a model to explain the formation of these films, in which the surface structure and reactivity of the mica substrate controls the orientation of the micellar precursor species, which in turn imposes order on the resulting solid film.

We report here on the synthesis of oriented mesoporous silica nucleation crystals and large-area continuous mesoporous silica films on mica, prepared under acid conditions using the following

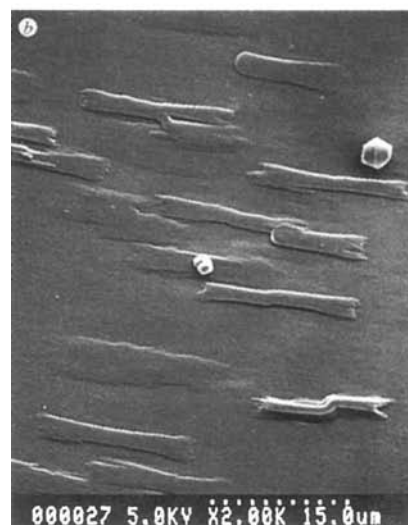
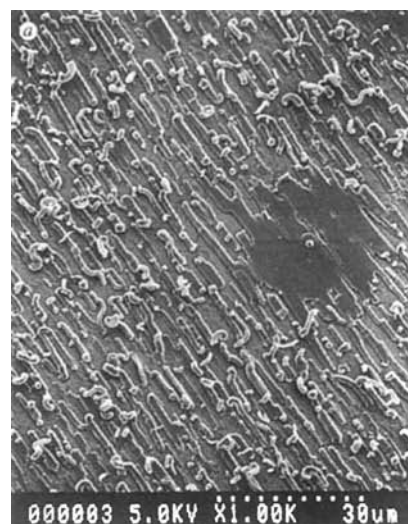


FIG. 1 SEM images of the evolution of an as-synthesized mesoporous silica thin film grown on mica. *a*, Micrograph showing the initial nucleation and growth of separate small oriented crystals with a preferred alignment and initiation of coalescence into a continuous film. *b*, micrograph of a mesoporous silica thin film after calcination. *c*, A boundary between two oriented domains in a large-area mesoporous silica thin film. (Dotted scale bars: *a*, 30 µm; *b*, 15.0 µm; *c*, 86 µm).

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reactant mole ratios: 100 H₂O : 7.4 HCl : 0.11 CTACl : 0.13–0.24 TEOS. Here CTACl is the cationic surfactant cetyltrimethylammonium chloride CH₃(CH₂)₁₅N(CH₃)₃Cl, and the TEOS (the silica source reagent) is tetraethylorthosilicate (C₂H₅O)₄Si. The aqueous–acid–surfactant synthesis mixture is prepared first, and TEOS is then added. The resultant mixture is stirred for 2–3 min at room temperature, and then transferred into a polypropylene bottle containing the freshly cleaved mica substrate. The substrate is arranged to lie horizontally in the synthesis vessel. The film is formed at 80 °C over a period of 1–2 h to 1 week.

Well formed mesoporous silica films from ~0.2 to 1.0 µm thick have been reproducibly grown from the bottom side of a horizontally held mica substrate. The as-synthesized films were washed with H₂O.

Scanning electron microscope (SEM) studies of the early stages of film formation reveal geometrical arrays of “elongated” micrometre-dimension mesoporous silica islands with a preferred growth direction (Fig. 1). These crystals grow in size and even-

tually coalesce to form a continuous mesoporous silica film. An SEM image of a calcined thin film (540 °C for 4 h in air) demonstrates that the macroscopic integrity of the film is maintained (Fig. 1b).

Calcined samples (weighing ~14 µg cm⁻² when 1,000 Å thick) preserve mesoporosity as indicated by a characteristic argon adsorption isotherm (Cahn balance) as well as Fourier-transform infrared (FT-IR) spectra of adsorbed benzene, perdeutero-benzene and tris-isopropylbenzene (not shown).

In the SEM and optical micrographs of the films, there is evidence of macroscopic crystalline domains which are preferentially co-aligned with the hexagonal unit cell axes of mica (Fig. 1c).

Transmission electron microscopy (TEM) lattice images of the as-synthesized and calcined films (epoxy-embedded and microtomed into 100–300 Å sections) show that the channels run predominantly parallel to the mica surface (Fig. 2). The ability to preserve the film on the mica substrate during the microtoming process implies good adhesion at the surface.

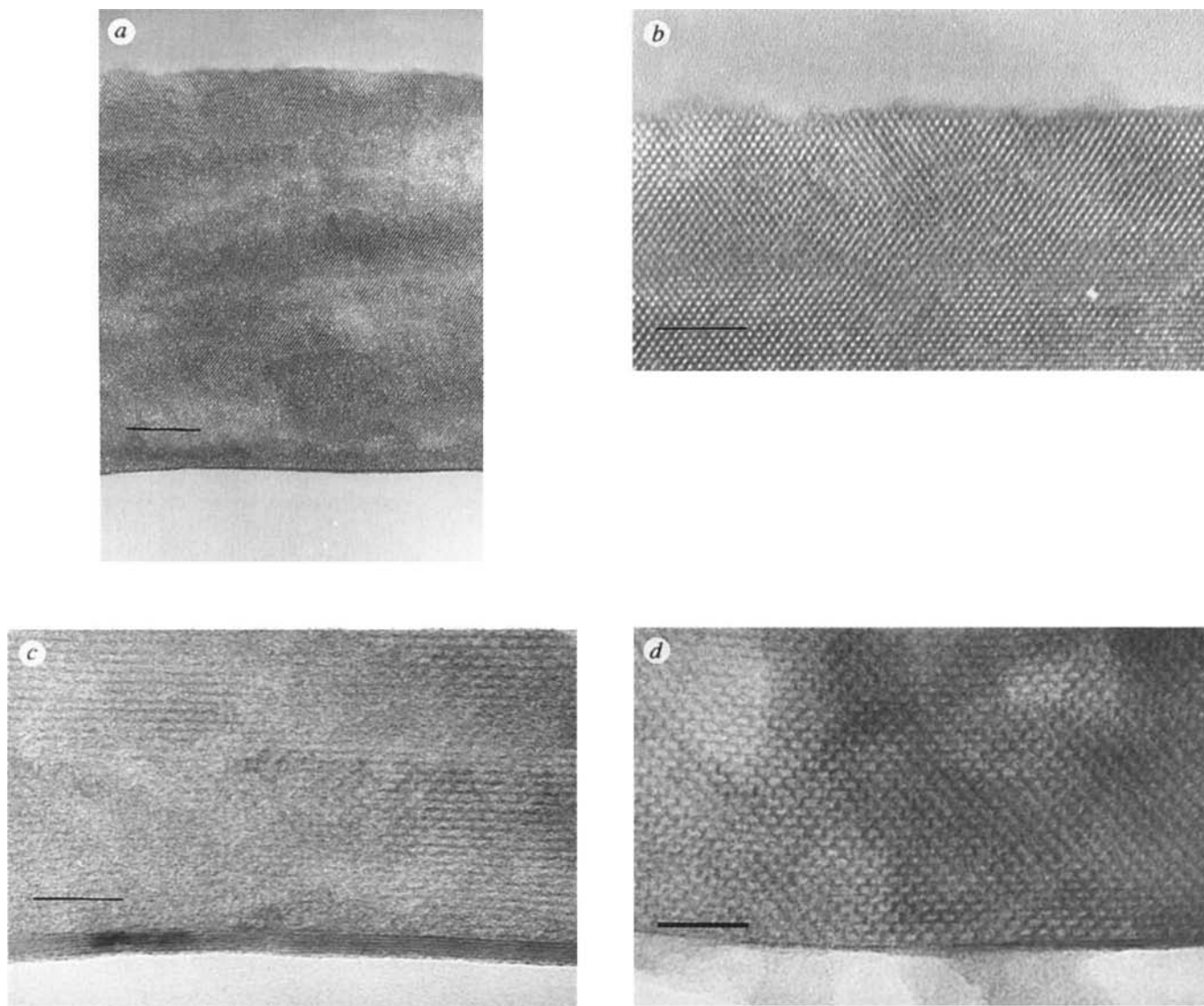


FIG. 2 TEM images of mesoporous silica films. a, As-synthesized (~5,000 Å thick), showing that the channels run predominantly parallel to the mica surface (scale bar, 100 nm); b, Transverse view of channels oriented parallel to the mica surface appears as a honeycomb pattern. The

edge profile reveals the “mesoscopic steps” on the surface of the film (scale bar, 50 nm); c, calcined, showing that mesopore formation commences at the mica surface (scale bar, 25 nm); d, depicting “brick-like” lateral distortions of the mesopores (scale bar, 25 nm).

TEM lattice images of the calcined films also convincingly demonstrate that channel formation commences at the mica interface (Fig. 2). Some TEM images have shown evidence of 'brick-like' lateral distortions of the mesopores. This deformation probably arises from the interfacial constraint of the film over extensive areas of the mica surface. The TEM observation of well-defined 'mesoscopic steps' on the external surface of the mesoporous silica film (Fig. 2) suggests that film formation involves multi-layer deposition of silica-surfactant micellar building-blocks.

The powder X-ray diffraction (PXRD) patterns for the as-synthesized and calcined mesoporous silica thin-film samples are shown in Fig. 3 (trace A and B respectively). We estimate the pores to be ~ 36 Å in diameter for the as-synthesized supported films with a 3–6 Å contraction following calcination. The PXRD patterns both reveal dominant (100) and (200) reflections, consistent with the TEM observations that the channels run predominantly parallel to the mica surface. Support for this proposal is obtained by comparison with the diffraction pattern from a randomly oriented mesoporous silica powder sample (Fig. 3, trace C). In this case the pattern displays the expected (100, 110, 200, 210) reflections^{1,2}. The absence of the (110, 210) reflections in the PXRD of the film samples indicates that the *c*-axis of the hexagonal unit cell is oriented parallel to the mica surface.

On calcination of the films, the anticipated contraction ($\Delta d_{100} = 2.5$ Å) of the hexagonal unit cell is observed, due to the removal of the surfactant template and accompanying condensation of SiOH groups^{1,2}. This provides compelling additional evidence that the originally deposited material is an oriented mesoporous silica. These oriented films can be removed intact from the mica substrate.

We propose a model whereby the formation of the mesoporous silica film begins with nucleation involving silica-surfactant-mica interfacial interactions that facilitate the assembly of silica-surfactant micellar seeds on the mica. When freshly cleaved, the $\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$ muscovite mica surface exposes aluminosilicate six-ring sites, whose structure and reactivity can be tailored by chemical procedures¹². Mica consists of double layers of tetrahedral $\text{SiO}_4/\text{AlO}_4$ building blocks sandwiching a single layer of octahedral AlO_6 units. The tetrahedra in a freshly cleaved mica surface are in the so-called down configuration, exposing an atomically flat hexagonal symmetry surface. Under acid conditions charge-balancing potassium ions are exchanged by protons, thereby providing reactive SiOH anchoring sites on the surface of the mica substrate for binding to silica-surfactant micellar precursor species.

Elongated mesoporous silica-film islands then grow on the surface, preferentially oriented along the hexagonal cell axes of mica. This implies that crystal growth is probably regulated by charge- and structure-matching at the interface.

The final stage involves the expansion in size and coalescence of the crystalline islands on the mica surface. Assembly of silica-surfactant micellar species on the established mesoporous silica layers results in the continued growth of the film.

This scheme for the formation of mesoporous silica films has much in common with biomineralization^{8,9}, and provides opportunities for the creation of new materials technologies^{13,14}. □

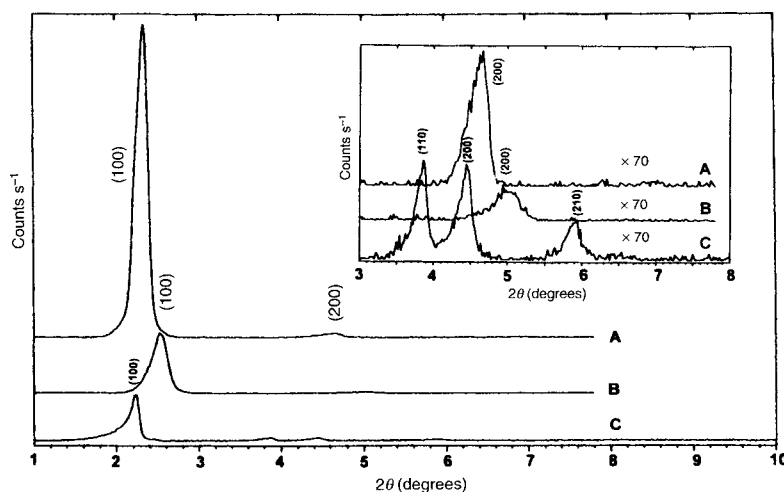


FIG. 3 The PXRD patterns for a mesoporous silica thin film grown on mica. Trace A, as-synthesized; trace B, calcined; trace C, mesoporous silica powder. Inset, PXRD reflections for $2\theta = 3^\circ$ – 8° with an intensity (counts per second) scale expansion of $\times 70$.

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Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes

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CLIMATE warming, acid deposition and increasing exposure to ultraviolet radiation are all regarded as widespread problems in boreal ecosystems. Here we report observations from twenty years of whole-lake acidification experiments, which show that these three problems are intimately linked. In our study area in northwestern Ontario, both climate warming and lake acidification led to declines in the dissolved organic carbon content of lake waters, allowing increased penetration of solar radiation. We suggest that some of the changes in aquatic ecosystems that have been attributed to lake acidification may in fact have involved increased exposure to ultraviolet light. Moreover, it seems that—

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particularly in clear, shallow lakes and streams—climate warming and/or acidification can be more effective than stratospheric ozone depletion in increasing the exposure of aquatic organisms to biologically effective UV-B radiation.

Our observations were made in the Experimental Lakes Area (ELA), northwestern Ontario, Canada, during the period 1971–90. During this 20-year period, climate warmed by an average of 1.6 °C (ref. 1). During this period, we performed several deliberate whole-lake acidification experiments at ELA, to elucidate the effects of acid deposition on boreal lakes². Long-term, ecosystem-scale measurements were made of meteorological, hydrological, chemical, physical and biological processes in both experimentally manipulated and reference ecosystems³.

Recently, there has been concern about the effects of stratospheric ozone depletion on increasing ultraviolet radiation. Incident UV-B radiation at the latitude of the ELA has increased by ~10% (refs 4, 5). Potentially harmful intensities of UV-B radiation can reach depths of several metres in clear freshwater lakes^{6,7}. Dramatic changes have been shown to occur in aquatic communities exposed to realistic intensities of UV-B^{8,9}, and photoinhibition of phytoplankton can occur to depths of several metres (ref. 10).

The penetration of UV-B in boreal lakes is known¹¹ to be a function of the concentration of dissolved organic carbon, DOC (Fig. 1). In most boreal lakes, DOC concentrations of several milligrams per litre are sufficient to provide an effective shield against ultraviolet radiation for aquatic organisms, restricting penetration of UV-B to a few decimetres. But, UV-B penetration increases exponentially as DOC declines. Two factors are responsible for the relationship: the proportion of colourless DOC produced in the lake increases in relative importance as DOC declines, and photobleaching and photodegradation of coloured DOC compounds increase as a function of residence time in the lake¹².

In situ plant production is the most important source of autochthonous DOC. Much of it is readily available for microbial metabolism^{13,14}. In contrast, DOC which enters lakes from terrestrial catchments usually contains highly coloured aromatic compounds that have largely resisted microbial decomposition in soils^{15,16}. In general the proportion of coloured DOC in boreal lakes increases as a function of the catchment/lake ratio^{17,18}.

The interactions between DOC and solar radiation are complex. DOC is the predominant factor attenuating the penetration of solar radiation of both visible and ultraviolet wavelengths in lakes of the boreal and other northern forested regions^{19,20}. As a result, it protects organisms from harmful ultraviolet wavelengths, and limits the depth to which photosynthesis can occur. It reacts

with UV-B radiation to form a number of chemically reactive and biologically toxic chemicals, including hydrogen peroxide, hydroxide radicals, carbon monoxide and superoxides, destroying, bleaching and chemically modifying DOC in the process^{21,22}. In many cases, the photolytic by-products of UV-B–DOC interactions are more available for microbial decomposition than the original molecules^{13,14}. The increased penetration of UV-B radiation and microbial breakdown can thus constitute a positive feedback, accelerating the removal of DOC.

Portable field instruments that accurately measure UV-B under water have only recently become available. In order to calculate past UV-B regimes in our lakes, we therefore used long-term measurements of DOC, and the relationship between DOC and UV-B shown in Fig. 1, which is derived from Canadian boreal lakes, including those at ELA. Data from the original publication¹¹ were refitted with a more complex negative exponential formula than originally used, which explained slightly more of the variance.

Our studies show that both climate warming and acidification cause declines in DOC, allowing increased penetration of solar radiation. Climate warming in summer is predicted by most global climate models to be particularly acute in boreal regions of North America^{23,24}. Although we do not know whether the observed 20-year warming trend is part of a natural cycle or induced by increasing emissions of greenhouse gases, our observations provide a useful glimpse of the consequences for boreal lakes. In addition to the observed increase in air temperature, precipitation decreased by 25%. Evapotranspiration increased by 35%, and once-permanent streams became intermittent. Increased forest fires, decreased chemical yields from catchments, clearer, warmer and chemically altered lakes, and a number of changes to the abundance, habitats and distribution of freshwater organisms were also observed^{1,25,26}.

Declines in DOC concentrations were among many effects of climate warming^{1,27}. The primary cause of the decline was the reduced export of DOC from terrestrial and wetland catchments to lakes as drought caused streamflows and water tables to decline. In-lake processes also played a role in the DOC decline²⁷.

During the 20 years, DOC in unperturbed reference lakes at ELA decreased by an average of 15–20%, allowing increases of 22–63% in the depth of UV-B isopleths of the lakes (Fig. 2a). As a result, the percentage of the lakes' volume exposed to any given intensity of UV-B increased by 60% for Lake 239 and ~22% for lakes 224 and 240 (Fig. 2b).

Acid deposition, caused by man-made emissions of oxides of sulphur and nitrogen, is probably the greatest threat of small boreal lakes in Canada and Eurasia²⁸. Although acidifying sulphur oxide emissions have been reduced by over 50% in Canada, and legislation is in place to compel similar reductions in the USA by early in the twenty-first century, these measures are estimated to have reduced the potential effect of acid precipitation on Canadian lakes by only about half²⁹.

In experimentally acidified lakes, DOC declined much more rapidly than in reference lakes²⁷. Widespread similar observations have been made in lakes acidified by acid rain^{30–32}.

In the most extreme acidification experiment at ELA, sulphuric acid was used to acidify Lake 302S from original pH values of 6.0–6.7 to pH 4.5 between 1980 and 1990. Acidification caused DOC to decline from original values of ~7.2 mg l⁻¹ to 1.4–1.6 mg l⁻¹, lower than in natural lakes of the area, and comparable to the clearest arctic and alpine lakes, or to some parts of the oceans. Less-acidified experimental lakes 223 and 302N (minimum pH 5.0 in both cases) showed substantial, but less extreme, declines in DOC. Even lower DOC values, as low as 0.5 mg l⁻¹, have been observed in acidified lakes near Sudbury, Ontario (ref. 33 and P. J. Dillon, personal communication).

The rapid decline in DOC caused by acidification during the period of record caused much greater increases in UV-B penetration than climate alone. In the most acidified lake, 302S, the depth of 1% UV-B penetration increased from ~0.3 m to over 2.8 m (Fig. 2a). As a result, the proportion of the lake's volume exposed

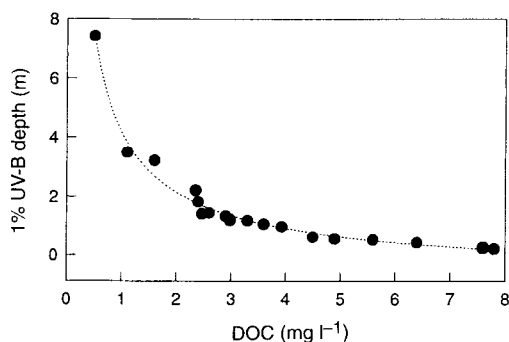


FIG. 1 The relationship between measured DOC concentration and the depth of the 1% UV-B isopleth. The dotted line represents the equation $1\% \text{ UV-B} = 5.173(\text{DOC})^{-0.706} - 1.029$, $r^2 = 0.98$, fitted to data from 18 lakes of boreal and northern Canada, including lakes in the ELA. This equation was used to calculate UV-B penetration from DOC concentrations measured between 1970 and 1990 in the ELA.

to >1% of incident UV-B increased over eightfold, from 6% to nearly 50% (Fig. 2b). The volume exposed to >1% of incident UV-B in Lake 223, which was acidified only to pH 5, increased by a factor of two. Other experimentally acidified lakes displayed similar increases in volume exposed to increased UV-B.

Greater effects of climate warming on transparency to UV-B would be expected in lakes that had lower original concentrations of DOC. For example, in Lake 224, where we have a shorter record (1974–90) for DOC, a decline of ~15% in DOC concentrations (which initially averaged $\sim 3.2 \text{ mg l}^{-1}$) caused an increase in the depth of 1% UV-B penetration of 24%, increasing the volume exposed to any given level of UV-B by 22%. Many large lakes of the area have comparably low DOC concentrations, and may have experienced similar changes in UV-B environments.

Low-DOC lakes are not rare in the boreal zone of North America. In boreal regions of Ontario, lakes with less than 3.6 mg l^{-1} DOC are about 20% of the total³³. The number of low-DOC lakes is higher in Quebec, but lower in the maritime provinces. Overall, we estimate that about 140,000 of the nearly 700,000 lakes in eastern Canada may have DOC concentrations low enough for UV-B penetration to be of concern. Low-DOC lakes are even more common in arctic, alpine and subalpine regions, where concentrations less than 1 mg l^{-1} are common. The highest concern must be for clear, shallow lakes, streams and ponds, where even modest declines in DOC may eliminate the small regions that are deep enough to provide refuges from damaging UV-B radiation. In particular, alpine areas may receive incident UV-B that is over 50% higher than at sea level. High-altitude species of trout have been shown to suffer sunburn patterns, increased fungal infections and higher mortalities at environmentally realistic exposures of UV-B³⁴.

It seems possible that some of the many changes to aquatic

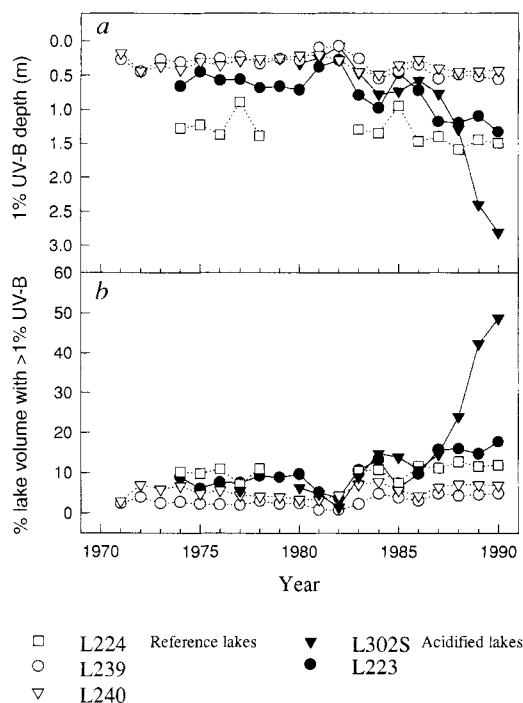


FIG. 2 a, Changes in the depth of 1% UV-B penetration in reference lakes 239, 240 and 224, during 20 years of warming and drought (1970–90), and in lakes 302S and 223, acidified during the period. UV-B penetration is calculated from DOC concentrations, using the equation in Fig. 1 legend. The depth of the 10% UV-B isopleth would be half the depth of the 1% value, and the relative change with time would be similar. b, The percentage of lake volume that was exposed to UV-B in excess of 1% of surface intensity, 1971–90.

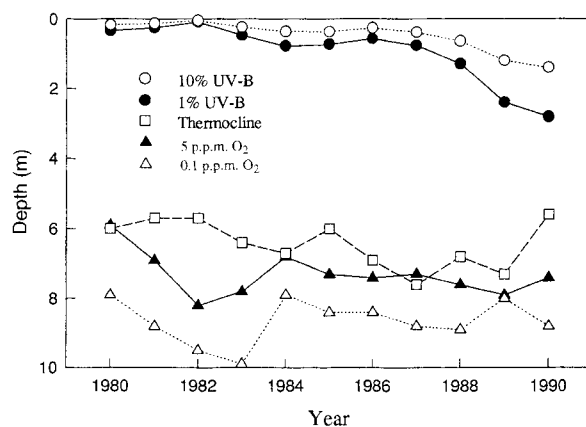


FIG. 3 Changes over time in the depth of UV-B light penetration, thermocline depth, and the zone of hypolimnetic hypoxia in Lake 302S, showing the potential for erosion of aquatic habitat by the combined effects of acidification, climate warming and UV-B exposure.

communities that have been attributed to lake acidification may have involved harmful UV-B exposure. The disappearance of species has usually been attributed to direct exposure to hydrogen ions, although indirect effects via food organisms, combined effects with aluminum or changes in parasite infestations have also been observed in studies of acidification^{35,36}. However, most species disappear in natural aquatic ecosystems at higher pH values than predicted by laboratory assays³⁷, suggesting that in ecosystems, additional stresses enhance the effects of acidification. It is possible that the increased exposure to UV-B caused by DOC decreases in acidified lakes is one such stress. In particular, non-motile littoral organisms in small lakes with shallow thermoclines like Lake 302S may be exposed to lethal intensities of UV-B. In midsummer, much of the warm upper layer of the lake is exposed to UV-B intensities that exceed of 10% of surface values. In addition to the stress of acidification, warm stenothermic (tolerant of a narrow range in temperatures) organisms might be confined to narrow strata just above the thermocline by high surface UV-B above and low oxygen below (Fig. 3).

Our results indicate that in aquatic ecosystems, climate warming and/or acidification can increase the exposure of organisms to UV-B much more than changes to incident UV-B caused by depletion of the stratospheric ozone layer. For example, in a lake containing 2 mg l^{-1} DOC, an increase of 10% in incident UV-B (such as that observed in the Northern Hemisphere) causes an increase of only 2.1% in the depth of 1% UV-B penetration. In comparison, a 10% reduction in DOC would cause an increase of 11% in UV-B penetration. The relative effects of DOC and incident UV-B would become even greater at lower concentrations of DOC. For example, an 80% decline in DOC, as in acidified lakes, would cause the depth of the 1% UV-B isopleth to increase by over 400%.

In clear oligotrophic lakes, the decreases in DOC caused by climate warming, drought and acidification should be of much more concern with respect to UV-B exposure than depletion of stratospheric ozone. In addition, the decline in DOC has other important effects. Increased penetration of total solar radiation causes thermocline deepening in small lakes^{1,20,38,39}. DOC is also important in chelation, flocculation and changes in mobility of trace metals and other chemicals^{32,40}. Clearly, the interactions of these three widespread problems in boreal regions deserve further study.

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The origin of harmonic tremor at Old Faithful geyser

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VOLCANIC eruptions are sometimes accompanied by a characteristic type of seismicity known as harmonic tremor, in which the signal is dominated by discrete vibration frequencies^{1–4}. This harmonic structure could reflect resonance behaviour in the excitation source^{4–6} or filtering of the seismic waves as they propagate through the surrounding rocks^{7–10}; but complexity and variability in the properties of volcanic systems make it difficult to discriminate between such mechanisms. To address this question, we have analysed the source and propagation characteristics of seismicity at Old Faithful geyser (Yellowstone National Park), the cyclic behaviour and accessibility of which make it an ideal natural laboratory for studying harmonic tremor associated with near-surface sources. We find that sharp pressure pulses inside the water column trigger distinct seismic events that give rise to a harmonic ground response whose frequency varies spatially but not temporally. A superposition of these seismic events creates the appearance of continuous harmonic tremor. The absence of resonance within the water column suggests that the harmonic motion must arise from the interaction of the seismic waves with heterogeneities in the surrounding elastic medium—most probably a near-surface soft layer.

Old Faithful is among the most studied geysers in the world. Located in the Upper Geyser basin of Yellowstone National Park, its surface expression is a 4-m-high, 60-m wide mound with an opening roughly $2 \times 1 \text{ m}^2$ at the top. The conduit extends downwards successively narrowing and opening into larger spaces, as described in ref. 11 and as inferred from spectacular video recordings made by J. Westphal, S. Kieffer and R. Hutchinson (personal communication). The geyser's eruptions are 2–5 min

long with the interval between them ranging from 30 to 100 min, and their time of occurrence is predictable to within 15 min.

The pioneer of scientific studies of the geyser was Rinehart^{12–14}, who was the first to deploy seismometers around the geyser and measure temperature inside it. Birch and Kennedy¹¹ continued with temperature measurements at different depths in the geyser, and Kieffer¹⁵ gave an elaborate description of the geyser's behaviour including its seismicity and thermodynamics. Kieffer¹⁵ first pointed out the similarities between geyser seismicity and volcanic seismicity, and the possible relevance of geyser studies to interpretation of volcanic tremor.

These studies provided a good overall understanding of geyser behaviour. But as all past studies used analogue recorders and short-period (~1-s period) seismometers without simultaneous time-resolved measurements of pressure and temperature, the source of tremor and its interaction with the solid medium at Old Faithful were not fully understood.

Our work at Old Faithful is designed to establish a cause-and-effect relationship between the source of seismic noise and the observed harmonic tremor. We have carried out three seismic studies at the geyser (1991, 1992 and 1994), as outlined in Fig. 1. We have measured time-resolved pressure variations inside the geyser, passive broadband and short-period seismicity around the geyser, and ground reaction to an external sledge-hammer source.

Figure 2 describes the details of a representative eruption cycle, the period between the end of one eruption and the end of the next. The activity is composed of single events with sharp onset and a decay time of 0.2–2 s depending on the station, which have the following characteristics: the frequency of the harmonic signals varies as a function of location; the harmonic signal is more pronounced in the horizontal components than the vertical component; the sledgehammer source produces spectral behaviour similar to the natural source; and the resonance frequency does not change with time (not shown in the figure).

A 30-s trace of the pressure sensors and the seismic record of the station BD60A is shown in Fig. 3a. The pressure pulses correlate well in time with the seismic events. Zooming in on one pulse in the record (Fig. 3b), we observe a distinct cause-and-effect relationship. Sharp pressure pulses are followed in less than 0.1 s by a seismic signal, with harmonic horizontal motion. When the individual events overlap, they appear on the seismic record as continuous harmonic tremor, as shown in Fig. 3c.

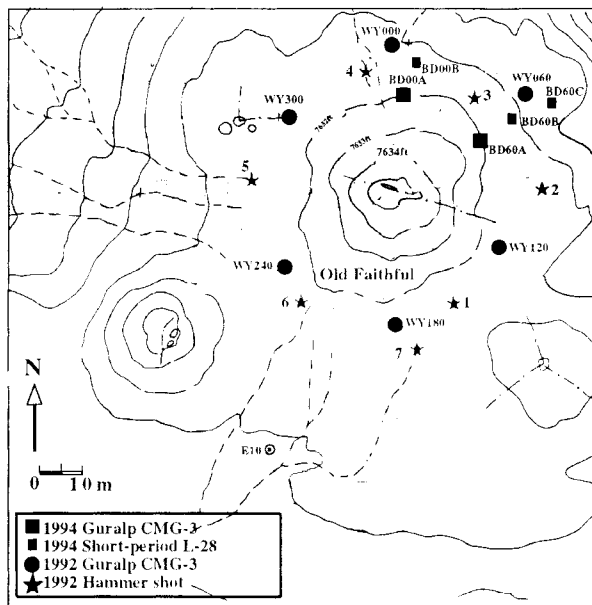


FIG. 1 Locations of three scientific excursions to Old Faithful geyser. The first was carried out in 1991 and deployed two broadband sensors (GURALP CMG-3ESP, flat velocity response from 0.0333 to 50 Hz) and REF-TEK six-channel portable data-loggers with a 16-bit digitizer. This served as a pilot study for a second and a more elaborate seismic survey, done in October 1992, which incorporated broadband recordings of geyser-generated signals and sledge-hammer pulses. Shown here is a map of the survey area and instrument setups for the 1992 and 1994 deployments in relation to Old Faithful and two of its neighbouring passive domes. The orientation of the geyser's orifice is indicated by the broken line across its dome. This survey included six broad-band setups identical to the 1991 instruments, which were placed around the geyser in different configurations for continuous recordings at 100 and 200 samples per second for periods of a day at a time. In addition an array of 96 short-period (natural frequency 1 Hz) vertical geophones was placed in a tight grid over the geyser's dome. On the basis of the observations from 1991 and 1992, a probe was designed and built to measure pressure inside the water column simultaneously with the seismic measurements on the geyser's dome. The instrument consisted of three sensors 3 m apart on a coaxial cable, each containing a KULITE XTC-190, high-frequency pressure transducer. The instrument was lowered into the geyser's conduit during the quiet period in a long eruption cycle until the bottom sensor was 22 m deep and below 7 m of water. The recording was made at 250 samples per second simultaneously with two broadband sensors and three 3-component short-period sensors (L-28, natural frequency 4.5 Hz) for 40 minutes. This last experiment (October 1994) obtained a direct measurement of the source of seismic waves and resolved propagation effects from source effects.

FIG. 2 *a*, A 2-hour-long sequence of vertical-component raw broadband velocity data recorded at station WY060, marked on Fig. 1, in 1992. Arrows indicating eruptions (E) bracket a 90-min long eruption cycle. A detailed discussion of eruption dynamics can be found in ref. 21. The general pattern of the eruption cycle starts with a quiet period. Then half an hour later, the activity increases in intensity and amplitude, and gradually decays until the final moments before an eruption. A short (~50-min) eruption cycle shows a similar behaviour without a period of seismic silence. The amplitude decay was explained by Kieffer¹⁵ by an increased acoustic impedance mismatch between the two phase water-steam mixture and the conduit walls, resulting from an increased amount of steam in the final stages of boiling. *b*, One minute of the record section indicated by arrow *b* in part *a* is blown up showing that the seismic activity in a consists of many distinct events. *c*, Three-component records of a single event recorded at the station WY120. 'Radial' and 'transverse' refer to the radial vector connecting the geyser and the sensor. Each component is shown with its Fourier spectrum. The horizontal components show a more harmonic behaviour than the vertical component. *d*, The radial components of the records from stations WY000, WY060 and WY120 with their Fourier spectra for the event shown in *c*. For comparison, hammer shot 4 (see Fig. 1) recorded at the same stations is shown with its spectrum (dashed curve). The same geyser event creates strong horizontal oscillations whose frequency varies from station to station. The hammer shot also generates harmonic horizontal motion with sharp spectral peaks. Most of the spectral peaks generated by the two different sources coincide. As the hammer shot is close to station WY000 it generates a generally 'white' spectrum at that site, but still excites one of the spectral peaks excited by the geyser. Some peaks do not coincide, probably due to the depth and location differences between the two sources.

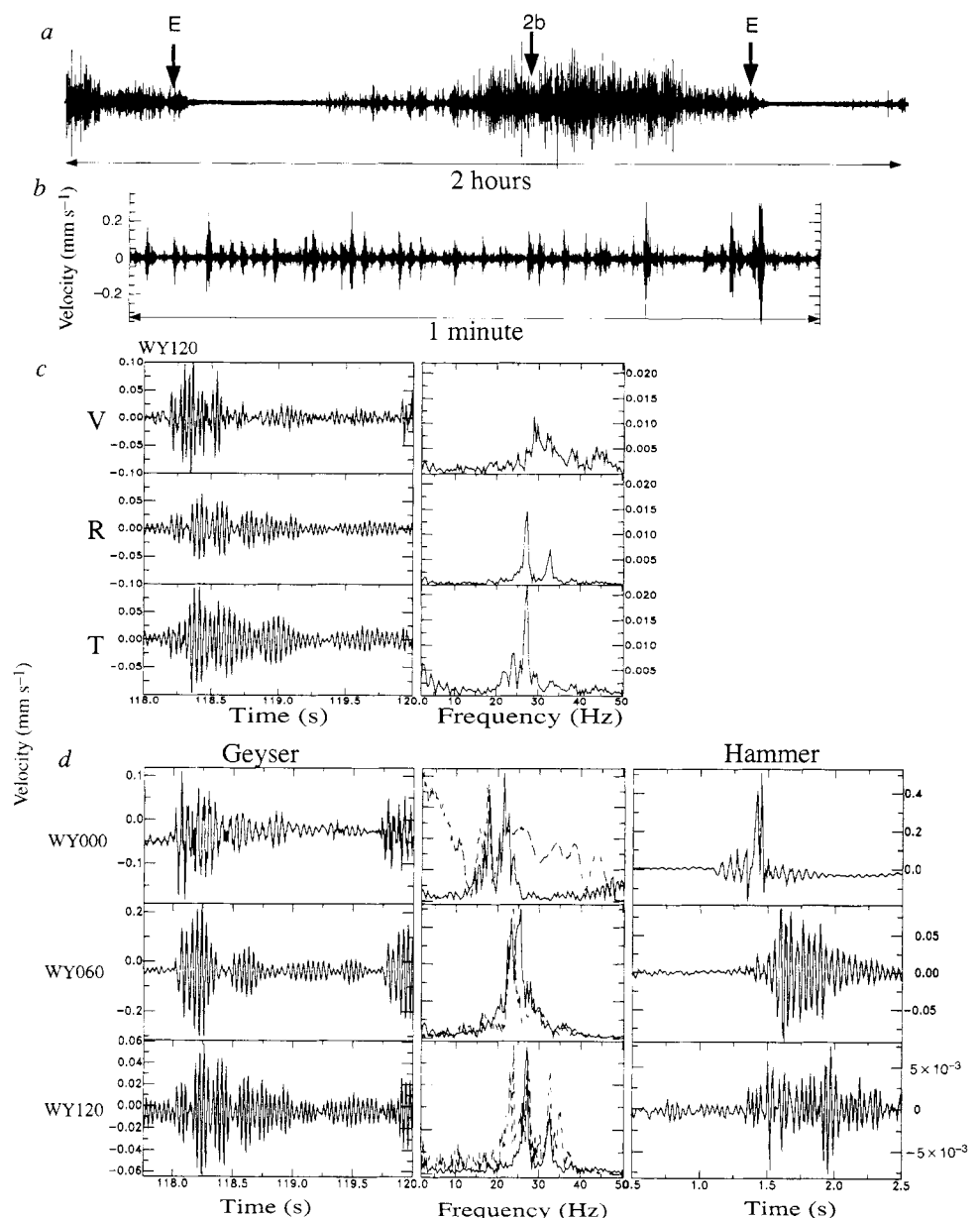


Figure 3 clearly demonstrates that the pressure source at Old Faithful geyser is impulsive, and that all of the harmonic resonance occurs in the medium between the source and the receiver. Harmonic tremor at Old Faithful is a 'path effect'. The lack of attenuation in the water column probably indicates an expected high absorption in a water–steam mixture¹⁶.

Analysis of arrival times of the pressure pulses at the three sensors indicates that most of the pressure pulses arrive at the top sensor first, implying that they originate near the top of the water column, in the coolest zone¹¹. The average arrival time difference between the top two transducers is $\sim 0.002 \text{ s} \pm 0.002 \text{ s}$ (that is, one sample period) which constrains the speed of sound in the two-phase water–steam fluid in the geyser to a lower bound of about $1,500 \text{ m s}^{-1}$, the speed of sound in water.

Although here we do not present a complete analysis of the physical process causing the pressure pulses, we point out that a likely source is bubble collapse as suggested previously^{15,17}. Also, pressure rebound, a common characteristic of bubble collapse, is occasionally observed (Fig. 3b, top trace). However, using the collapse of an evacuated spherical cavity¹⁸ to determine the order of magnitude of bubble size from observed collapse times ($\sim 10 \text{ ms}$) yields an impossibly large ($\sim 1 \text{ m}$) diameter. This result indicates that another type of process, such as the sudden

flash of superheated water into steam, is responsible for the pressure pulses.

How does the elastic rock surrounding the conduit convert an impulsive input into a harmonic output? One possibility is that a soft layer of thickness H and a particularly low shear (S) velocity, β , acts as a local shear-wave resonator (Fig. 4a). Part of the compressional (P) wave energy excited by the impulsive pressure change is converted to shear-vertical (SV) waves when incident upon the bottom of the soft layer. As the S wave in the layer is much slower than the P wave in the subterranean medium, the S wave travels nearly vertically in the layer, thereby producing reverberations with frequency $f = \beta/4H$, equal to the observed frequency, 25 Hz, for the structure shown in Fig. 4a. Some SV energy in turn leaks into the subterranean medium, so the reverberation decays with time. On the other hand, the P wave in the layer is not much slower than in the lower medium, so it transmits directly out without reverberating. It has been demonstrated¹⁰ that a low-velocity zone underlying the ice sheet at Mount St Helens created a similar effect.

Because the wavelength of the S waves, $\lambda = (\beta/f) = 2 \text{ m}$, is small, only the local structure near the site of the observations controls the frequency. Changes of the layered structure take place between seismic stations, so the resonant frequency varies

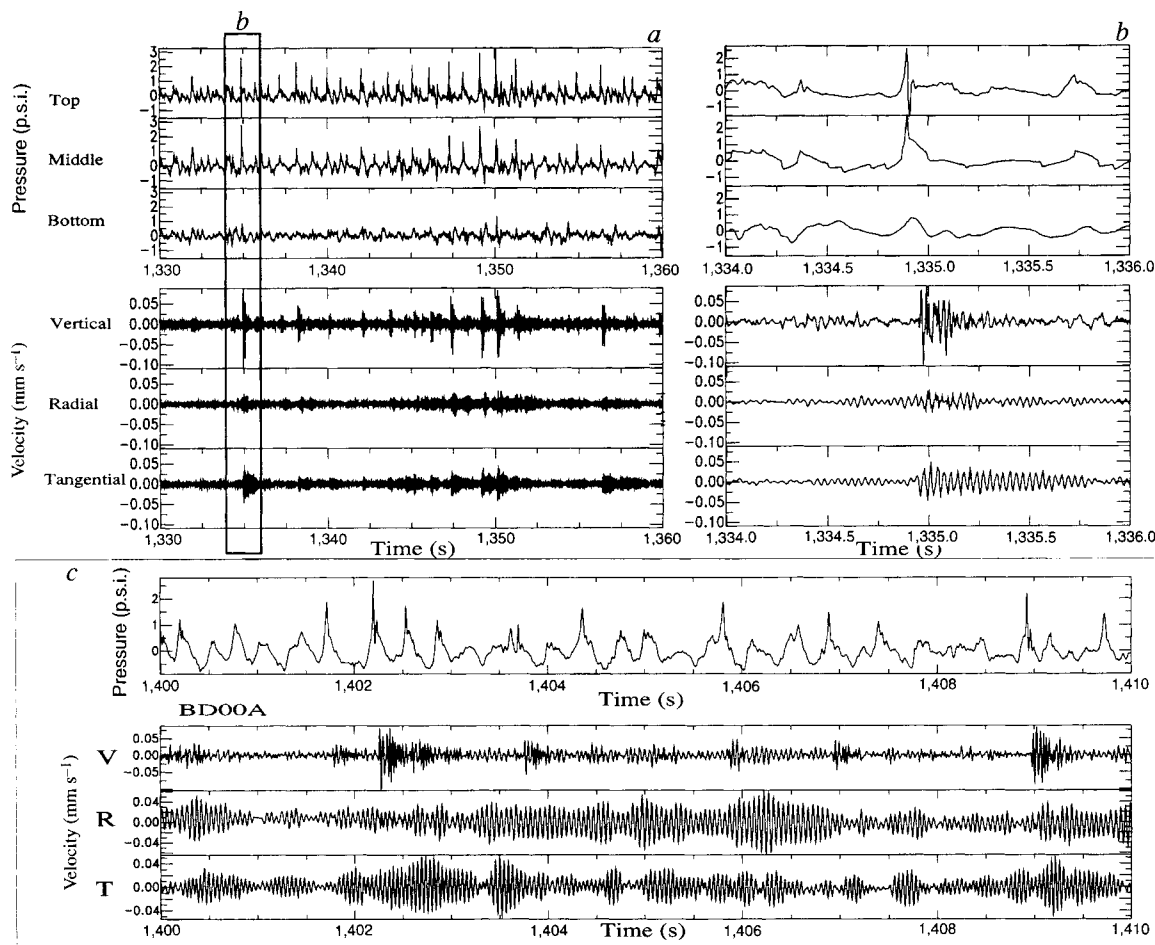
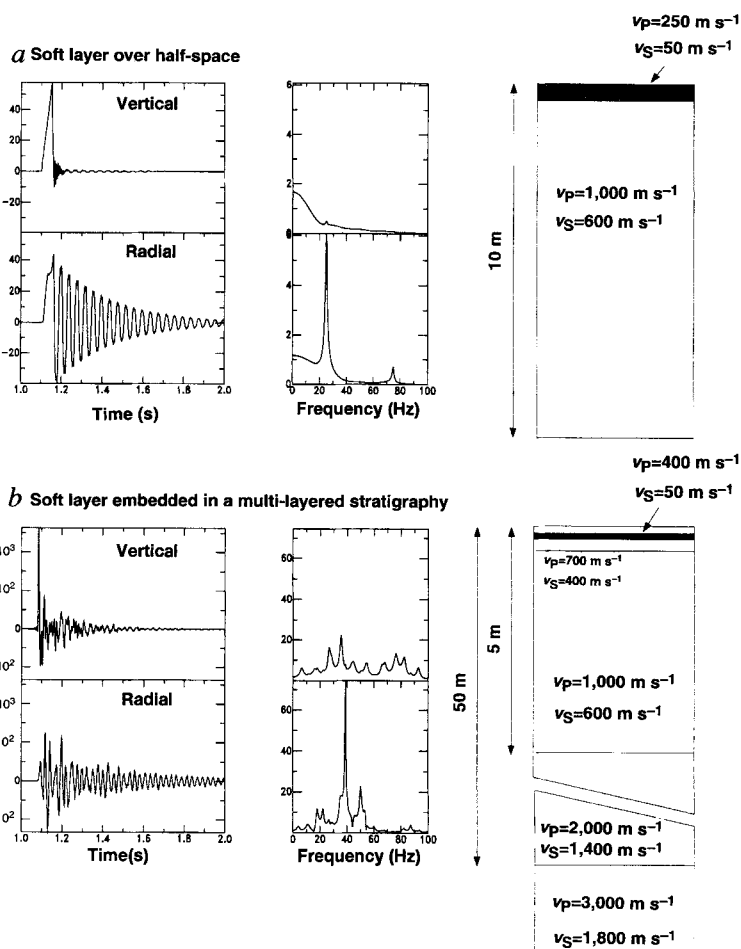


FIG. 3 *a*, Simultaneous pressure records (high-pass filtered at 1 Hz) and seismic traces (station BD60A), 30 s long, showing a direct correspondence between the pressure pulses and the seismic signals that follow them. During the 40-min-long underwater measurement, moisture leaked into the sensor containers. As a consequence, the lower pressure transducer suffered large drift on a $\sim 5 \text{ min}$ timescale, and its high-frequency response also seemed to have been degraded. For the same reason, attempts to measure time-averaged temperature were

unsuccessful. *b*, A 2-s portion of the record shown in part *a*. A single pulse excites seismic waves. The horizontal components show a strong harmonic behaviour. *c*, A 10-s time window displaying the record of the top pressure transducer and the broadband record BD60. Individual pulses in the water column superimpose to form a continuous harmonic seismic wave-train. It has been suggested that in volcanic areas individual harmonic events ('B-type' events) compose the continuous volcanic tremor.

FIG. 4 a, A synthetic seismogram and its Fourier spectrum generated using Haskell's propagator matrix method²² illustrate the response of a soft layer over a half-space to a P plane wave from below at an angle of 30° . A 0.5-m-thick soft layer with P velocity of 250 m s^{-1} and S velocity of 50 m s^{-1} is underlain by a faster medium. (These velocities are reasonable for unconsolidated, water-saturated soil^{23,24}). Some of the P energy is converted to shear-vertical (SV) polarized energy upon passing through a plane separating two layers. As a result of the strong shear velocity impedance mismatch between the layer and its surroundings, little SV energy leaks out of that layer, whose thickness modulates the frequency of oscillation, observed on the radial component in this case. At the same time, the vertical component, which is more sensitive to compressional motion in this configuration, will not be as harmonic as a result of the P energy leaking out of the layer because of the milder compressional impedance mismatch. This model can easily account for the variation in frequency from site to site by a slight change in the soft layer thickness. b, A more complex model allows the P waves to leak out of the layer and reflect back, whereas the S energy is predominantly trapped in and above the soft layer. In this case the P waves reflect from several boundaries in the geological structure and back to the surface, resulting in a longer nonharmonic wave-train for the vertical component, whereas the soft layer still traps the shear energy as in the simpler case.



from site to site. A more complicated structure with several layers results in a behaviour that is closer to that observed (Fig. 4b). It should be noted that shallow soft layers are observed in a cross-section of a dome neighbouring Old Faithful.

An intriguing observation is the strong tangential, or shear-horizontal (SH) motion shown in Fig. 2c. If the source is indeed an explosion or implosion, it contains mostly compressional energy. An explosion occurring within a vertical crack can, however, generate SH motion²⁵. Another explanation for the strong SH motion is coupling of radial and tangential motion. A likely coupling mechanism is propagation through an interface whose intersection with the wave-plane is not horizontal.

What can harmonic tremor at a geyser teach us about volcanoes? The prevailing view of volcanic harmonic tremor is that it is caused by resonance at the source, and many models have been proposed to account for the observed spectral peaks^{2-6,19}. But owing to the simple harmonic behaviour of the ground motion,

source-resonator models are necessarily inconclusive about the excitation mechanism. Any resonator (be it an organ pipe, an oscillating crack or a waveguide) may be excited by a number of different mechanisms, provided they have the right frequency content. In volcanoes particularly, the physical properties needed for determining the source dimensions and source properties are poorly constrained. For example, the sound velocity in a two-phase fluid mixture can vary by two orders of magnitude, from a few tens of metres per second to a few thousands of metres per second²⁰, and the elastic wave velocity in rock can vary from $\sim 200 \text{ m s}^{-1}$ in unconsolidated ash to $\sim 2,000 \text{ m s}^{-1}$ in lava flows, both of which are abundant on volcanoes. Further, most volcanic edifices have extremely complicated stratigraphies, which complicate path effects. The observations at Old Faithful imply that it would be difficult to resolve the physics of the source through seismic observations alone, without identifying the path effects and integrating them into the analysis of volcanic tremor. $\square$

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Non-chondritic platinum-group element ratios in the Earth's mantle

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THE Earth's upper mantle has an overabundance of highly siderophile elements (platinum-group elements, gold and rhenium), relative to what would be expected from equilibrium with the metallic core¹⁻³. This excess is now widely believed to have been introduced as a "late veneer" by meteorite bombardment during early Earth history, but after separation of the core⁴. Here we report high-precision analyses of platinum-group elements (PGEs) from fertile upper-mantle lherzolites, which show variations in relative abundances that exceed those in the chondritic meteorites that are thought to have furnished the late veneer⁵. In particular, the Pd/Ir ratio, at about 1.76, is significantly greater than that of known chondritic meteorites⁶. Our results, combined with previous indications of both non-chondritic and nearly chondritic PGE ratios in the few other fertile mantle lherzolites that have been analysed^{2,3,7-11}, strongly suggest that the mantle is heterogeneous in its PGE content on scales of ~100 kilometres. If the observed PGE ratios can be reconciled with a plausible meteoritic source, the observed heterogeneity may reflect an original spatial variation in the late veneer; otherwise, an additional fractionation mechanism seems to be required.

Until now, the commonly accepted PGE content of the Earth's mantle has been defined mainly from two elements (Ir and Pd) analysed in a few tens of basalt-hosted spinel lherzolite xenoliths and tectonically emplaced orogenic lherzolite massifs^{1-3,7,12}. Information on the other PGEs usually comes from primitive lavas formed by partial melting of the mantle (komatiites, picrites or basalts). These latter generally have Pd/Ir and Pd/Pt ratios greater than chondritic. But the manner in which this PGE signature pertains to the mantle remains unclear, as the PGEs fractionate during partial melting because of large differences of compatibility between Pd, Pt and Ir (refs 8, 13-16).

In the present study we have determined the contents of Ir, Ru, Rh, Pt, Pd and Au in 14 upper-mantle lherzolites using inductively coupled plasma mass spectrometry, ICP-MS (Table 1). These samples have been collected over a distance of 200 km in the small (<3 km in maximum dimensions) orogenic spinel lherzolite massifs tectonically emplaced into the northern part of the Pyrenean range, in southwestern France. All but one sample are fertile lherzolites (11-16% clinopyroxene) that are depleted in light rare-earth elements, LREE (refs 17-19). ($\text{La/Yb}_N < 0.6$; $+9 < \epsilon_{Nd} < +14$; $-23 < \epsilon_{Sr} < -31$). Two samples (TUR 7, DES 7) have Al_2O_3 contents akin to primitive mantle estimates (Table 1). Such compositions indicate very limited degrees (<7%) of partial melting, dated at ~2 Gyr by Re-Os systematics²⁰. Pyrenean lherzolites are either devoid of hydrous minerals (for example, FON B) or contain very small amounts of disseminated Ti-pargasite¹⁷. Caussou sample 70-5 is unusual because of its high Ti-pargasite content (18 wt%) and TiO_2 - and LREE-enrichments (Table 1) which were caused by pervasive re-equilibration with alkali basalts¹⁷⁻¹⁹.

The absolute abundance of each PGE varies very little within a single sample and is generally very similar to those previously

TABLE 1 Analyses of Pyrenean orogenic spinel lherzolites

Sample	Geographical location*	LOI (wt%)	Al_2O_3 (wt%)	TiO_2 (wt%)	La/Yb_N	Ni†	Cu†	S†	Ir‡
TUR 7	Turon de Técoière (Western Pyrénées)	0.07	4.18	0.18	0.34	1,925	36	270	2.62
DES 7	Tuc d'Esse (Central Pyrénées)	1.5	4.05	0.17	0.74	1,910	n.a.	280	2.80
70-354	Sem (Eastern Pyrénées)	0.5	3.80	0.14	0.83	2,000	31	170	2.56
TUR 23	Turon de Técoière	1.8	3.70	0.14	n.a.	1,955	36	220	3.23
86 V2	Lherz (Eastern Pyrénées)	1.0	3.58	0.21	0.62	1,831	22	250	2.70
DES 1	Tuc d'Esse	1.4	3.45	0.1	1.4	1,910	43	210	3.64
71-336	Freychinède (Eastern Pyrénées)	0.69	3.47	0.15	0.24	2,040	29	179	2.60
FON B	Fontête Rouge (Eastern Pyrénées)	0.02	3.23	0.12	0.3	1,960	31	200	2.92
MON 2	Moncaup (Central Pyrénées)	1.1	3.20	0.12	0.23	2,054	29	150	3.66
70-5	Caussou (Eastern Pyrénées)	0.99	3.18	0.76	1.54	n.a.	26	160	3.82
71-321	Lherz	0.96	3.10	0.12	0.39	2,020	n.a.	220	3.62
71-324	Lherz	2.9	2.96	0.13	0.40	1,990	26	170	2.59
POR 1	Portetery (Eastern Pyrénées)	0.14	2.85	0.08	0.20	2,095	29	190	2.78
PCOU 1	Pic Couder (Eastern Pyrénées)	0.45	2.83	0.10	0.56	2,035	30	210	2.66
Average									3.0
CI-normalized average									±0.5
Separated sulphides									0.0062
Values for CI chondrites ³⁵									±0.0009
Rock standards									1,207.2
SARM7 (measured)									481
(certified)									71
									74
									±12
WGB1 (measured)									0.22
(certified)									0.33§
Blanks (average on three complete blank runs)									0.04
Detection limits									±0.014
									0.04

Sources of data: LOI, Al_2O_3 , TiO_2 and La/Yb_N mostly from refs 17-19. (N indicates CI-chondrite-normalized); Ni, Cu, S mostly from ref. 34; all PGE analyses were done at the University of Montpellier using a VG 353 ICP-MS. Each analysis was performed on a 15-g aliquot after NiS fire-assay and Te co-precipitation. Accuracy and precision were evaluated by analyses of the international rock standards SARM7 and WGB1; the reproducibility has been estimated by replicate analyses of samples 71-336, 71-321 and 71-324 (average of 4 analyses) and FON B (average of 11 analyses); n.a., not analysed; LOI, loss on ignition.

reported for mantle lherzolites (Table 1). The 11 replicate analyses of sample FON B yield relative standard deviations (standard deviation/mean) ranging between 3.8 and 8%. Likewise, the variations of PGE contents from one massif to another do not exceed 30%. In order to locate these elements at the hand-sample scale, 15 mg of Fe–Ni–Cu sulphides from sample 86-V2 were extracted with a magnetic separator and purified by hand picking under a binocular microscope, then analysed by ICP-MS. Gold and the PGEs are up to 600 times more concentrated in the separated sulphide than in the whole rock, supporting earlier conclusions that the main carriers of PGE in mantle lherzolites are sulphide microphases².

The Pyrenean lherzolites have CI-chondrite-normalized PGE patterns characterized by a slight but perceptible fractionation between heavy platinoids (Ir, Pt) and light platinoids (Ru, Rh and Pd; Fig. 1). Pd, Ru and Rh contents vary from 0.007 to $0.012 \times$ CI chondrite, with Ir and Pt contents falling in the range 0.006 – $0.009 \times$ CI chondrite. Accordingly, the ratios of Pd to Ir (mean, 1.76 ± 0.2) and Pd to Pt (mean 0.83 ± 0.09) are higher than chondritic (Table 1). Such higher-than-chondritic Pd/Ir and Pd/Pt ratios have been duplicated by a completely different analytical method (radiochemical neutron activation) or using another ICP-MS instrument (Fig. 2). They are observed not only in the LREE-enriched metasomatized lherzolites (for example, 70-5) but also in the LREE-depleted lherzolite from the Fontête Rouge massif, which suggests that the siderophile and lithophile trace elements behave independently. Likewise, there is no correlation with Al_2O_3 contents which could suggest a possible effect of the small melting degrees on the PGE patterns. Ru/Ir and Rh/Ir ratios are also higher than chondritic, whereas Pt/Ir ratios are approximately chondritic. The Au contents agree fairly well with previous

neutron activation determinations²³. Palladium to gold ratios show random variations around the chondritic value, which reflect both the poorer precision of ICP-MS analyses of gold (30–40%) and intrinsic variations in the context of this element in the Pyrenean lherzolites²¹. The fractionated CI-normalized PGE patterns of whole rocks are faithfully reflected in the pattern of the separated sulphides (Fig. 1). The sulphides are even more fractionated, as they have higher Pd/Ir, Rh/Ir and Pd/Pt ratios than the silicates that host them (2.68 versus 1.76, 0.52 versus 0.33, and 1.38 versus 0.8, respectively). The same observation has been made on crustal mafic rocks and interpreted in terms of stronger partitioning of Pd and Rh into base metal sulphides, compared to the other PGEs²².

Knowledge of whether the non-chondritic mantle sampled by the Pyrenean lherzolites is depleted in Ir and Pt or enriched in Pd and Rh primarily depends on the poor PGE database existing for fertile, upper-mantle lherzolites. These show a much larger scatter than the Pyrenean lherzolites (Fig. 2). A part of this scatter, especially that observed within a single locality, is probably inherent in the analytical imprecision. Most of the previous analyses were performed on very restricted amounts of rock powder (usually less than 5 g) which generates 'nugget effects' because of the highly inhomogeneous distribution of PGE microphases²³. Figure 2 clearly demonstrates the improvements that ICP-MS analyses performed on 15-g aliquots bring to the precision of PGE determinations. Beyond analytical uncertainties, however, Fig. 2 suggests that the mantle does not have a homogeneous PGE signature on a global scale. Most of the Kilbourne Hole (Western USA) lherzolite xenoliths analysed by Morgan *et al.*³ are identical in composition to the Pyrenean samples. This is also the case for the few lherzolites analysed in

Ru‡	Rh‡	Pt‡	Pd‡	Au‡	Pd/Ir	Pd/Pt	Pt/Ir
5.0	0.96	5.6	5.3	1.1	2.02	0.95	2.14
7.3	0.93	5.8	5.4	1.4	1.93	0.93	2.07
6.3	0.99	5.6	5.1	0.7	1.99	0.91	2.19
4.9	0.98	6.7	5.3	1.0	1.64	0.79	2.07
5.4	0.88	5.5	4.0	0.7	1.48	0.73	2.04
4.7	1.07	7.7	5.7	0.9	1.56	0.74	2.11
4.4	0.93	5.7	4.7	1.1	1.81	0.82	2.19
±0.2	±0.04	±0.7	±0.45	±0.4			
5.9	1.05	6.2	5.4	1.0	1.85	0.87	2.12
±0.4	±0.1	±0.5	±0.3	±0.3			
5.3	1.07	7.2	5.6	0.6	1.53	0.78	1.97
8.9	1.32	8.6	6.9	n.a.	1.81	0.80	2.25
5.8	1.20	7.2	5.6	–	1.55	0.78	1.99
	±0.01	±0.01	±0.1				
5.1	0.95	6.0	5.3	1.3	2.05	0.88	2.32
	±0.05	±0.4	±0.7				
6.6	1.06	6.4	5.3	0.8	1.91	0.83	2.30
6.1	1.25	6.7	5.7	1.2	2.14	0.85	2.52
6.0	1.04	6.5	5.4	1.0			
±1.2	±0.1	±0.9	±0.6	±0.3			
0.0083	0.0077	0.0065	0.0096	0.0070			
±0.0016	±0.0009	±0.0008	±0.011	±0.0017			
3,114.5	625.1	2,335.1	3,239.1	605.0	2.68	1.39	1.93
712	134	990	560	140	1.16	0.56	2.06
380	222	3,716	1,503	277	21.17	0.40	52.34
430	240	3,740	1,530	310	20.67	0.41	50.54
±57	±13	±45	±32	±15			
0.26	0.16	5.3	12.6	2.1	57.27	2.38	24.09
0.3§	0.3§	6.1	13.9	2.9	42.12§	2.28	18.48§
		±1.6	±2.1	±1.1			
0.78	0.009	0.15	0.12	0.7			
±0.07	±0.003	±0.03	±0.03	±0.1			
0.2	0.01	0.1	0.1	0.34			

* From ref. 17.

† p.p.m.

‡ p.p.b.

§ Provisional.

the Zabargad orogenic lherzolite massif (Red Sea)⁷. The Baldissero and Balmuccia orogenic lherzolites (Italy) and some lherzolitic samples from the Bay of Islands ophiolitic complex (Newfoundland) also show higher-than-chondritic Pd/Pt ratios^{9,10}. Meanwhile, however, numerous lherzolite xenoliths from all around the world (Cochize Crater; Western USA; Nunivak Island, Northern USA; Mt Quincan, Australia; Thaba Putsoa and Matsoku, Lesotho) have been reported to be almost chondritic^{3,5}. In spite of the large scatter of individual data points, the average Pd/Ir of nine spinel lherzolite xenoliths from Victoria Tertiary basalts (Australia) is, within error limits, almost chondritic² (Fig. 2a). This is also true for some international peridotite standards collected in ophiolitic complexes (PCC1, California; DZE, China)¹¹ and for the three Ronda (Spain) orogenic lherzolites analysed so far⁸.

As for absolute abundances, the mantle lherzolite nodules that give nearly CI-chondritic Pd/Ir ratios generally are lower in Pd than Pyrenean lherzolites (Fig. 2). For example, the mean Pd contents of Victorian spinel lherzolite xenoliths is 3.8 p.p.b. (ref. 2), a result recently confirmed by 18 further analyses by radiochemical neutron activation²⁴. A similar Pd content (3.6 p.p.b.) can be extrapolated for the Archaean mantle from the fairly constant Ti/Pd ratio (3×10^5 ; ref. 25) of Archaean komatiites, assuming that the primitive mantle contained about 1,100 p.p.m. Ti (Ref. 26). Compared to these values, the Kilbourne Hole non CI-chondritic xenoliths and orogenic lherzolite massifs are definitely Pd-enriched. The mean Pd contents increase

from the Pyrenean massifs (5.4 p.p.b.), to Ronda (5.5 p.p.b.), Kilbourne Hole (5.5 p.p.b.), Zabargad (5.8 p.p.b.) and Ivrea Verbano massifs (7.6 p.p.b.)^{3,7,8,9}. Even higher Pd contents (>10 p.p.b.) have been reported for the Lanzo orogenic lherzolites, Italy²⁷. By contrast, there is no significant difference between the mean Ir content (3 p.p.b.) of the 14 Pyrenean lherzolites analysed here and the average Ir content (3.5 ± 0.8 p.p.b.) calculated from published Ir analyses^{1-3,5,7,12}.

From the present study of Pyrenean lherzolite massifs, it seems very likely that the Pd and probably Rh contents of the terrestrial mantle may vary significantly on spatial scales of ~100 kilometres. This conclusion contradicts interpretations that postulate a general Pd enrichment in the mantle through (1) less efficient extraction of this element from the Earth's proto-mantle by the core formation process³ or (2) separation of ~1% of Pd-rich metal from the liquid outer core and mixing with the primordial mantle immediately after core formation²⁵. In addition, the first mechanism would have fractionated the mantle too efficiently because of the strong differences between D_{Pd} (10^7) on the one hand and D_{Ir} and D_{Pt} on the other (10^{12} ; D is the Nernst partition coefficient between iron metal and silicate liquid)^{28,29}. Even in the most favourable case, namely, formation of the core at very high temperature (3,500 K), the Earth's proto-mantle would have Pd/Ir and Pd/Pt ratios two orders of magnitude higher than those of the Pyrenean orogenic lherzolites (116 versus 1.8 and 500 versus 0.8, respectively). The second hypothesis is based on the assumption that the liquid outer core shows a fractionated Pd/Ir ratio relative to chondrites, which is, to date, highly debated³⁰.

Although the observed Pd- (and also probably Rh-) heterogeneity in the mantle does not contradict the late veneer hypothesis, it should reflect either an original variation in the chondrite composition or fractionation mechanisms inside the mantle post-dating the meteoritic bombardment. There are a few enstatite chondrites which have Pd/Ir ratios matching the lower ratios of the Pyrenean lherzolites (1.56 versus 1.48)^{31,32}. However, the mean Pd/Ir ratio of undifferentiated meteorite groups is clearly lower than the Pyrenean lherzolites⁶. Furthermore an enstatite chondritic component would have delivered too much Au compared to what is present in these rocks. Alternatively, the Pd-rich mantle reservoir can be explained by incorporation of very limited amounts (<0.1%) of metallic core material after the late veneer or by some Fe-Ni-Cu sulphide accumulation. Plume-related oceanic island basalts that may have incorporated some core material in their mantle source are richer in PGEs than other lavas^{13,33}. In contrast to the model suggested by McDonough²⁵, core-mantle

exchange should have occurred at periodic stages during the history of the mantle to explain the scale of the Pd-rich mantle and to take into account the post-Archaeon age of Pyrenean orogenic lherzolites²⁰. Of course, as mentioned above, the Pd-enrichment of the mantle can be explained by this process only if the outer core is itself Pd-enriched. The sulphide accumulation hypothesis, already suggested for some orogenic lherzolites²⁷ is strongly supported by the observation that Cu-Fe-Ni sulphides concentrate Pd more than any of the other PGEs. In addition, all of the Pd-rich lherzolites including the Kilbourne Hole xenoliths have significantly higher sulphur contents than the basalt-borne lherzolite xenoliths that indicate nearly chondritic PGE ratios (150–250 p.p.m. versus <50 p.p.m. S; Table 1)^{2,7,9,34}. At present,

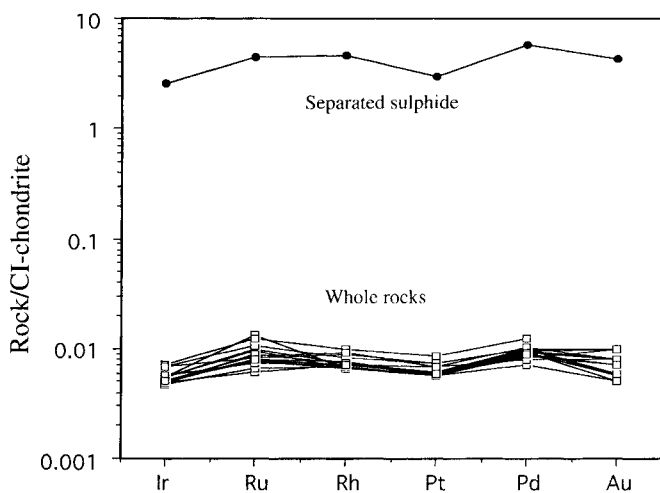


FIG. 1 CI-chondrite-normalized PGE patterns of Pyrenean orogenic spinel lherzolites. Open squares, whole rocks; filled circles, sulphides separated from sample 86 V2. Normalizing values after ref. 6.

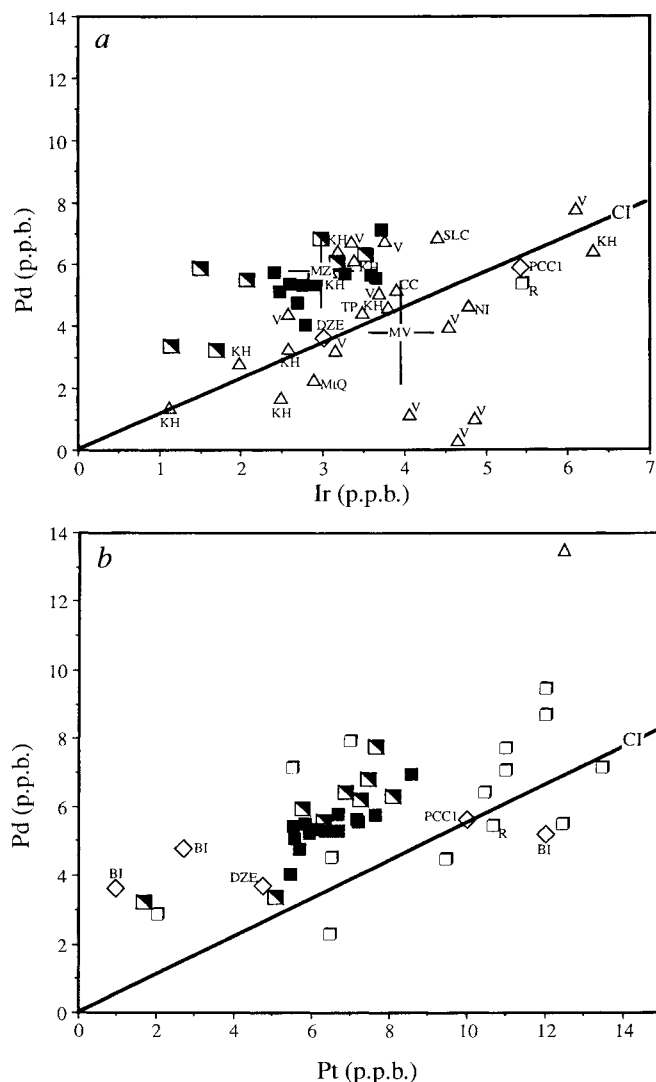


FIG. 2 Pd versus Ir (a) and Pd versus Pt (b) in Pyrenean lherzolites, and comparison with literature data. Filled squares, Pyrenean lherzolites (this study); half-filled squares, unpublished ICP-MS and radiochemical neutron activation analyses of Pyrenean lherzolites; open squares, other orogenic lherzolites (R, Ronda, Spain⁸; other data, Baldissero and Balmuccia, Italy⁹). MZ, mean Pd/Ir ratio and standard deviation of five spinel lherzolites from Zabargad, Red Sea⁷. Open diamonds, ophiolitic lherzolites (BI, Bay of Islands, Newfoundland¹⁰; DZE and PCC1: peridotite standards from China and USA¹¹). Open triangles, spinel lherzolite xenoliths from alkali volcanics (KH, Kilbourne Hole, Western USA^{2,3,5}; V, Victoria Newer Basalts, Southern Australia²; CC, Cochise Crater, Western USA³; MtQ, Mont Quincan, Australia³; NI, Nunivak Island, Northern USA³; TP, Thaba Putsoa, Lesotho³; SLC, Salt Lake Crater, Hawaii³). MV, Mean Pd/Ir ratio and standard deviation of the nine lherzolites from Victoria Newer Basalts²; CI, CI chondrite ratios after ref. 6.

none of the mechanisms discussed above can be ruled out. Clarification of this question will require more high-quality PGE analyses coupled with sulphur data, allowing for rigorous comparison as well as determination of the lateral and vertical extent of the high-Pd mantle. □

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Fossil evidence for the origin of the marsupial pattern of tooth replacement

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EXTANT marsupials are distinctive in their pattern of dental development¹, in that only one tooth is replaced postnatally in each jaw. Interpretation of this pattern for marsupials ancestrally is disputed^{2–5}, partly because ontogenetic data in fossils have been unobtainable. Here we present an ultra-high-resolution X-ray computed tomography (CT) study of the tiny fossil *Alphadon*, which represents the first evidence of dental development and replacement in a Mesozoic marsupial. In the known pattern of tooth replacement and development, *Alphadon* is identical to living marsupials, a derived similarity suggesting that this pattern is ancestral to Marsupialia, and that it was established by the Late Cretaceous, at least. This pattern has been correlated with some specialized aspects of marsupial lactation^{1,6}. Hence the presence of a marsupial pattern of tooth replacement in *Alphadon* provides indirect evidence that at least some specialized features of marsupial reproductive processes arose during the Mesozoic.

The adult dentition of primitive living marsupials includes three premolars and four molars, a configuration known to date to the Early Cretaceous⁷ and thought to result from a derived developmental pattern within Theria¹. Of these tooth positions, the last premolar locus (conventionally termed P3) is the only one in the entire dentition at which an erupted deciduous tooth is replaced postnatally by its successor. The homologies of the marsupial dentition are not universally accepted^{2–5}, and dispute usually

involves ontogenetic evidence. The adult dentition is thought to result from a unique developmental pattern¹, involving succession of vestigial teeth at incisor and canine loci, retardation and non-replacement of deciduous first (dP1) and second (dP2) premolars, normal replacement of a deciduous (dP3) by a successor (P3) tooth at the last premolar locus, and normal differentiation of the molars (M1–4). Thus the anterior two premolars of marsupials appear to be unreplaced deciduous teeth. Until now, little was known of the antiquity of the marsupial tooth-replacement pattern. Single jaws bearing presumed dP3 have been reported from the Late Cretaceous⁸ and Paleocene^{5,9}, but definitive ontogenetic corroboration has been unobtainable.

We have extracted ontogenetic data from an exceptionally

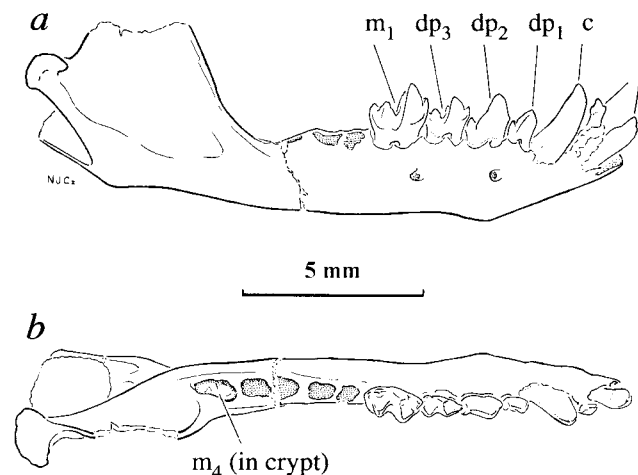


FIG. 1 *Alphadon* sp. (OMNH 27380), left mandible (reversed to correspond with CT images in Figs 2 and 4) from the Upper Cretaceous part of the North Horn Formation, Utah¹⁹. *Alphadon* is a relatively long-lived, generalized taxon²⁰; its contained species are distinguished primarily on the basis of upper molar features^{2,8}, so species referral of the specimen is uncertain. The specimen includes I_{2–37}, the canine (c), dP_{1–2} (conventionally termed P_{1–2}), a molariform dP₃, M₁, alveoli for M_{2–3}, and a partly exposed crypt for M₄; the presence of dP₃ and the pre-eruptive stage of development of the last molar indicate that it is from a juvenile individual. a, Labial, and b, occlusal views.

complete mandible of the Cretaceous marsupial *Alphadon* (Fig. 1), using an ultra-high-resolution X-ray CT scanner (Fig. 2). Comparison was made to a dated developmental series of the small didelphid *Monodelphis domestica*, based on sectioned osteological specimens (Fig. 3), histological sections, and cleared and stained material, and to the small dasyurid *Sminthopsis virginiae*, based on histological sections (Fig. 4).

In dental development, *Alphadon* corresponds closely to living marsupials, which enables estimation of its developmental stage and indicates a tooth-replacement pattern identical in all observable details to the relatively stereotyped, distinctive pattern that characterizes Recent marsupials. The most significant developmental data are the degree of eruption and root formation of the canine, the pre-eruptive stage of the replacement P_3 , and the degree of development and rotation of the last molar, all of which correspond to the dentition of *Monodelphis domestica* at 60–70 days of age. The developing P_3 of *Alphadon* lies lingual and deep to the erupted dP_3 , as is the common pattern among mammals. Living marsupials are somewhat variable in the exact timing and spatial relationships of the developing P_3 ; in *Alphadon*, as in *M. domestica* and *S. virginiae*, it lies beneath the anterior root of dP_3 , which undergoes some early resorption. The developing P_3 of *Alphadon* is higher in the jaw than in 60–70-day-old *M. domestica*, in this respect more closely resembling the 81-day *S. virginiae* (Fig. 4). In *S. virginiae*, P_3 begins erupting between 102–124 days, after M_4 has completely erupted (W.P.L. and P. Woolley, unpublished data); a similar pattern of late P_3 eruption also characterizes *Monodelphis* and most extant didelphid genera. A greater degree of calcification for M_4 than for P_3 in the juvenile *Alphadon* dentary is consistent with a comparable pattern of late eruption in the

Cretaceous taxon. It is unlikely that tooth succession occurred at the anterior two premolar loci of *Alphadon* because there is no evidence of developing teeth in these positions, and the implanted teeth are completely erupted, with fully formed roots.

Many features commonly used to diagnose Marsupialia as monophyletic are either plesiomorphic, controversial or not observable in fossils¹⁰. The presence of a marsupial pattern of premolar replacement in *Alphadon* suggests that this character complex is diagnostic of the last common ancestor of *Alphadon* and living marsupials. Recent phylogenetic analyses differ on whether *Alphadon* is a basal marsupial or a proximate sister-taxon of marsupials within a more inclusive taxon, Metatheria (see refs 4, 11, 12). As yet there is no evidence, direct or indirect, bearing on the pattern of tooth replacement in deltatheroidans¹³ or other putative fossil metatherians which have a postcanine tooth formula similar to that of marsupials (contrary to ref. 13). Despite this comparatively minor phylogenetic equivocation, it seems clear that the pattern of premolar replacement so distinctive of marsupials among living mammals arose during the Mesozoic.

The most distinctive characteristics of living marsupials are related to their reproductive biology, which is associated with birth of extremely altricial young and subsequent development during a period of extended maternal lactation^{14–16}. Particularly characteristic of marsupial lactation is the 'period of fixation' during which young are continuously attached to teats for a prolonged period¹⁷. The highly modified pattern of replacement in the anterior dentition of marsupials has long been linked to developmental constraints imposed by the 'period of fixation'^{1,6}. Neither the replacement pattern nor, by implication, nipple fixation is

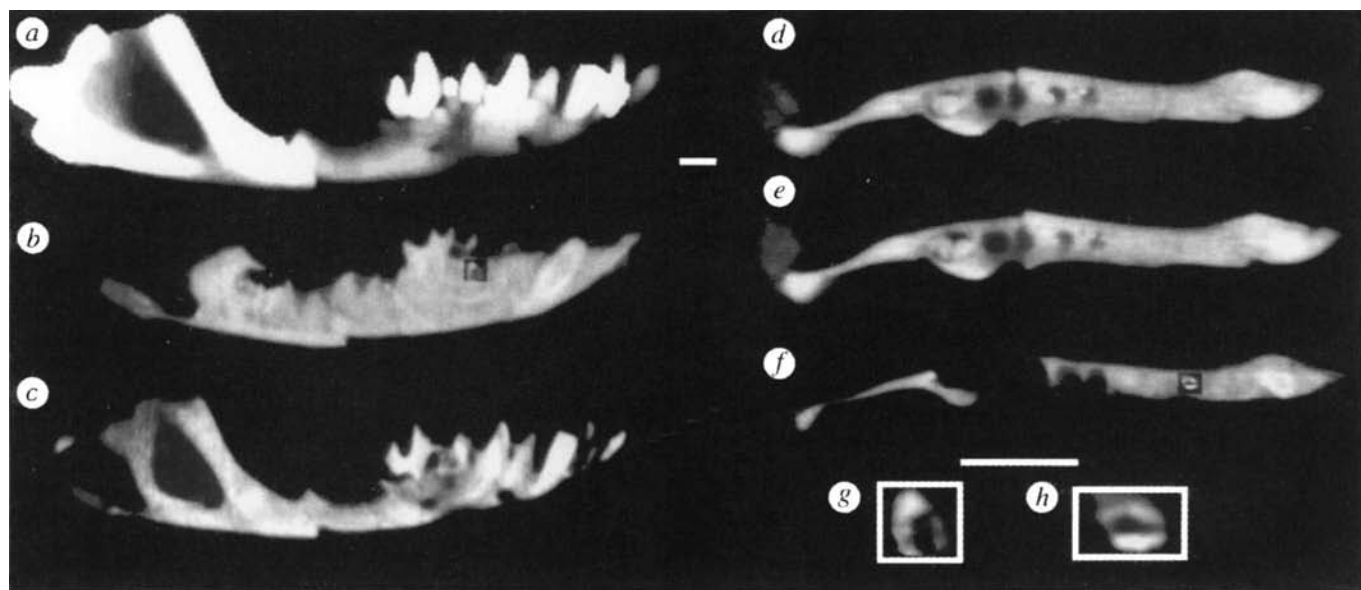


FIG. 2 CT scans of left mandible, *Alphadon* sp. (OMNH 27380). Examination of complete image sequences reveals that the canine has achieved nearly complete eruption and root formation; there is no evidence of unerupted teeth in the incisor/canine region, and we conclude that the erupted, rooted teeth at these positions represent true successors, as they are in extant marsupials¹. a–c, g, Parasagittal sections; d–f, h, horizontal (occlusal) sections. a, Composite reconstruction of 123 parasagittal sections; note dark area, indicating poor ossification, in the dentary below dP_3 . b, Section through the lingual part of the jaw, showing canine root, calcified protoconid of P_3 (in box below dP_3 ; see magnified inset, g), and part of the trigonid calcification for M_4 . c, More labial section, showing space of poor ossification below dP_3 and mandibular foramen. d, Occlusal view showing base of M_4 trigonid. Also visible are the alveoli for dP_3 , suggesting partial resorption of the roots. e, More dorsal section, showing some differentiation

of trigonid cusps of M_4 and, possibly, a rudiment of its talonid. f, More dorsal section, showing the calcified protoconid (in box; see magnified inset, h) of P_3 , somewhat lingually placed in the dentary. g, Magnified view of box in b, showing parasagittal section through calcification of developing P_3 . h, Magnified view of box in f, showing coronal section through calcification of developing P_3 . Scale bars, 1 mm.

METHODS. Data were obtained with an industrial scanner capable of two orders of magnitude greater resolution than conventional diagnostic scanners²¹; for this tiny specimen, it was configured with a 120 Kv micro-focal X-ray source and a CCD detector that resolved the specimen into 123 parasagittal, 253 horizontal and 468 frontal sections taken at 18- μ m (parasagittal, horizontal) and 36- μ m (frontal) intervals. Consecutive sections were rendered into digital animations viewable on personal computers, following a protocol described elsewhere²¹.

FIG. 3 Partial developmental series of the extant didelphid *Monodelphis domestica*: sectioned osteological specimens of right mandible. Each dated individual is represented by two parasagittal sections: left, region of M_4 ; right, selected views of the antemolar dentition. The following is a synopsis of relevant features and/or changes in the series (those not included in the figure are enclosed in parentheses). Day 51, canine in early eruption; pulp cavity broadly open with no root formation. (No macroscopic evidence for successor P_3 ; dP_3 roots complete.) Roots of M_3 short, broadly open to mandibular canal. M_4 calcification limited to protoconid, rotated about 30° . Day 57, canine somewhat more erupted, root beginning to form. Small, dorsally placed crypt for successor P_3 present under anterior part of dP_3 , containing P_3 in presumed bell stage; no calcification evident. Roots of M_3 longer, but not extending into mandibular canal. M_4 with all trigonid cusps distinct, although possibly separate in part; trigonid rotated about 45° . Day 65, canine mostly erupted, root nearly complete. Chamber for successor P_3 enlarged, ventrally placed, containing well-developed protoconid cap with early calcification; chamber extends dorsally along lingual margin of anterior root of dP_3 . Roots of M_3 extend into mandibular canal. M_4 with small heel; rotation unchanged. Day 70, canine erupted with fully formed but open root. Successor P_3 broader based (some resorption of dP_3 roots apparent). Roots of M_3 more elongate. M_4 talonid complete; rotation of tooth reduced to about 30° . Day 75, canine development appears complete. Protoconid of successor P_3 taller, better calcified. Day 85, both roots of dP_3 show clear resorption; chamber of P_3 more dorsoventrally extended, containing larger, better-calcified P_3 . Roots of M_3 extend completely through mandibular canal. M_4 with both roots fully formed; rotation reduced to about 8° .

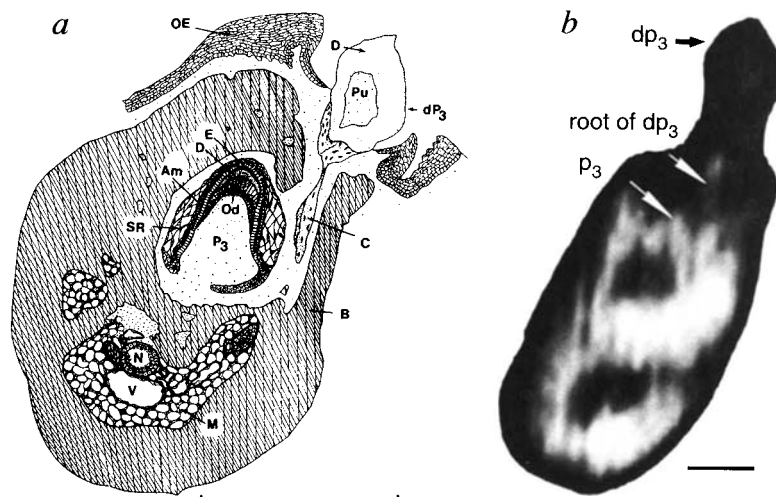
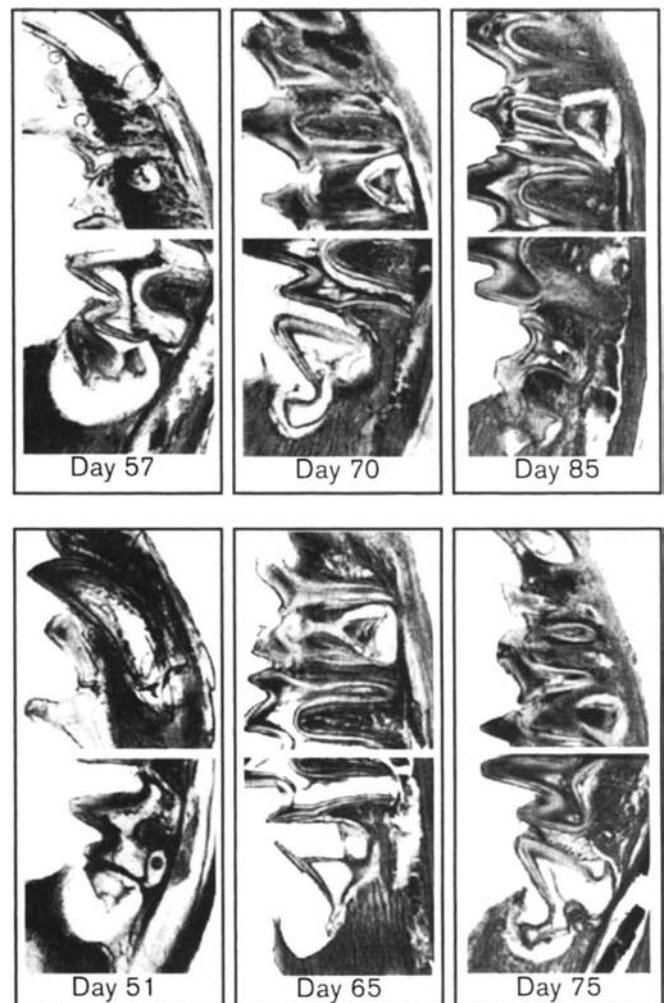


FIG. 4 Comparison of *a*, *Sminthopsis virginiae*, based on histological section of 81-day juvenile, and *b*, *Alphadon* sp. (OMNH 27380), based on CT section. Both sections are in the frontal plane, taken through the dP_3 and developing P_3 . In both, dP_3 and part of its anterior root, labial to the P_3 , can be seen; also shown are the mandibular canal, housing a neurovascular bundle while the animal was alive, and the calcification for the developing P_3 , near the top of an unossified space within the dentary. In the histological section, most of the anterior root of dP_3 is out of the plane of section; only root cementum (C) is seen. Only the apex of the P_3 protoconid is calcified, and it is capped by a moderately thick layer of dentin (D) and a thin layer of enamel (E). A well-developed neurovascular bundle, composed of nerve fibres (N), venules (V) and small arterioles, is partly enveloped by spongy marrow (M) deep to the developing P_3 . Other abbreviations: Am, ameloblasts; B, alveolar bone; Od, odontoblasts; OE, oral epithelium; Pu, pulp; SR, stellate reticulum. Scale bar, 1 mm.

likely to have characterized the common therian ancestor of marsupials and eutherians^{1,18} because the latter are primitive in replacing most or all of the antemolar dentition. Although characteristics of the reproductive system cannot be observed directly in fossils, the modified developmental pattern in the anterior dentition of marsupials provides indirect evidence of nipple fixation¹. Thus the developmental pattern seen in *Alphadon* suggests that at least some reproductive specializations of marsupials, including nipple fixation, were probably established

during the Mesozoic, earlier than previously suggested¹⁵. Other aspects of the ancestral therian and metatherian reproductive patterns remain to be elucidated. □

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Productivity and sustainability influenced by biodiversity in grassland ecosystems

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THE functioning and sustainability of ecosystems may depend on their biological diversity^{1–8}. Elton's⁹ hypothesis that more diverse ecosystems are more stable has received much attention^{1,3,6,7,10–14}, but Darwin's proposal¹⁵ that more diverse plant communities are more productive, and the related conjectures^{4,5,16,17} that they have lower nutrient losses and more sustainable soils, are less well studied^{4–6,8,17,18}. Here we use a well-replicated field experiment, in which species diversity was directly controlled, to show that ecosystem productivity in 147 grassland plots increased significantly with plant biodiversity. Moreover, the main limiting nutrient, soil mineral nitrogen, was utilized more completely when there was a greater diversity of species, leading to lower leaching loss of nitrogen from these ecosystems. Similarly, in nearby native grassland, plant productivity and soil nitrogen utilization increased with increasing plant species richness. This supports the diversity–productivity and diversity–sustainability hypotheses. Our results demonstrate that the loss of species threatens ecosystem functioning and sustainability.

The diversity–productivity hypothesis is based on the assumption that interspecific differences in the use of resources by plants allow more diverse plant communities to utilize more fully limiting resources and thus attain greater productivity^{6,8,17,18}. A related hypothesis is that nutrient leaching losses from ecosystems should be a decreasing function of plant diversity because of greater nutrient capture and/or immobilization in more diverse ecosystems^{4,5,17}. Taken together, these lead to the diversity–sustainability hypothesis; that the sustainability of soil nutrient cycles and thus of soil fertility depends on biodiversity. But the relationships between biodiversity and ecosystem functioning remain controversial^{3,10,13,18–23} because existing field data are from studies that lacked direct experimental control of biodiversity or sufficient replication. Only direct control of diversity allows attribution of responses to diversity rather than to other correlated factors^{8,18,20–23}. Moreover, the species composition of plots must be

randomly determined to avoid biases caused by the traits of particular species^{20,21}.

Here we report results of a field experiment in which the number of plant species was experimentally controlled (Fig. 1 legend). Our 147 plots, located on nitrogen-limited soil²⁴, were planted with either 1, 2, 4, 6, 8, 12, or 24 species. The species assigned to each plot were chosen by a separate random draw of the appropriate number of species from a pool of 24 North American prairie species. The impacts of diversity on plant productivity, nutrient capture and nutrient leaching were observed during the second year of growth. We also sampled a native grassland to determine the relationships between these variables in an undisturbed mature ecosystem.

Treatments created an experimental biodiversity gradient. Plant species richness, Shannon diversity (H'), and effective species richness (e^H ; Fig. 1a) were all significantly correlated with the number of species seeded into the plots (Pearson's $r = 0.81$, $r = 0.74$, $r = 0.75$, respectively; $n = 147$, $P < 0.001$ for all). Two measures of peak standing crop (our estimate of plant productivity) were positively correlated with the species-richness treatment (total plant cover: $r = 0.39$, $n = 147$, $P < 0.001$, Fig. 1b; biomass estimated by light penetration: $r = 0.27$, $n = 147$, $P < 0.001$). Both estimates were similarly dependent on observed plot species richness ($r_{\text{cover}} = 0.55$, $r_{\text{biomass}} = 0.42$, $n = 147$, $P < 0.001$ for both) and effective species richness ($r_{\text{cover}} = 0.29$, $r_{\text{biomass}} = 0.29$, $n = 147$, $P < 0.001$ for both). Thus, greater plant diversity led to greater productivity during the second year of ecosystem establishment.

Rooting-zone extractable soil NO_3^- was a decreasing function of species richness (Fig. 1c). Extractable NH_4^+ had a similar pattern ($r = -0.18$, $n = 147$, $P = 0.03$). This indicates that more species-rich plots more fully utilized soil mineral nitrogen, the main limiting resource²⁴. Below the rooting zone, soil NO_3^- concentrations

TABLE 1 Factors influencing plant total cover (productivity) in the biodiversity experiment

Variable	Parameter	Student's <i>t</i>	<i>P</i>
Intercept	18.9	3.82	0.0002
Species richness	1.31	6.72	0.0001
Rooting zone NO_3^-	−26.6	−2.20	0.03
Rooting zone NH_4^+	7.82	1.65	0.10
Root mass	2.96	5.00	0.0001

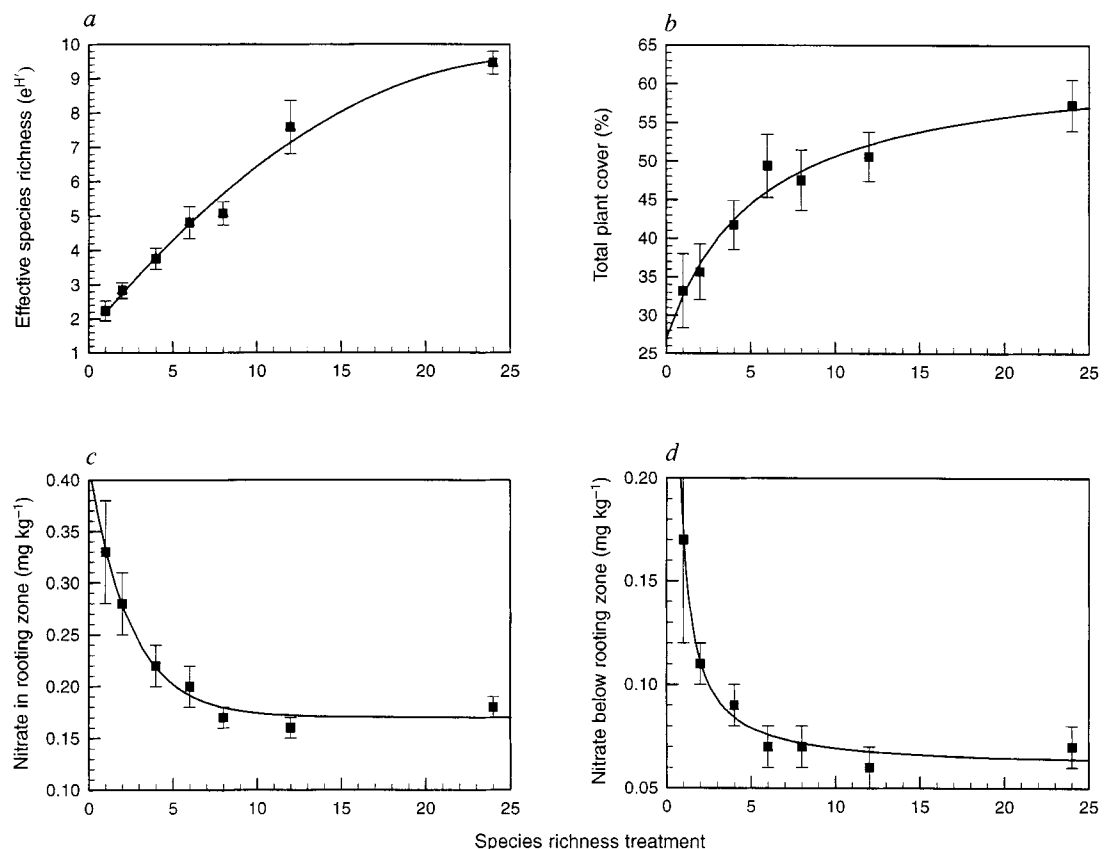
Multiple regression of total plant cover (dependent variable) on listed variables, each measured in all 147 plots. Overall $F_{4,142} = 28.5$, $P < 0.0001$, $R^2 = 0.45$.

TABLE 2 Factors influencing soil NO_3^- in the rooting zone in the biodiversity experiment

Variable	Parameter	Student's <i>t</i>	<i>P</i>
Intercept	0.33	12.0	0.0001
Species richness	−0.005	−2.74	0.007
Plant total cover	−0.001	−1.56	0.12
Root mass	0.003	0.14	0.89

Multiple regression of rooting zone soil NO_3^- on listed variables, each measured in all 147 plots. Overall $F_{3,143} = 7.39$, $P < 0.0001$, $R^2 = 0.13$.

FIG. 1 a, Effective species richness is $e^{H'}$, where H' is the Shannon diversity index; $e^{H'}$ is the number of equally abundant species required to give the observed H' . Curve is a simple second-order fit ($R^2 = 0.60$, $n = 147$, $P < 0.001$). b, Total plant cover is the sum of cover of all vascular species in a plot. Fitted curve is $y = 27 + 36.4x/(5.48 + x)$, $R^2 = 0.18$, $n = 147$, $P < 0.001$. c, Soil nitrate (as mg of N per kg of dry soil) at 0–20 cm depth, where 79.8% of roots occur. Fitted curve is $y = 0.17 + 0.24e^{-0.41x}$, $R^2 = 0.22$, $n = 147$, $P < 0.001$. d, Soil nitrate (as N) at 40–60 cm depth, where only 6.5% of roots occur. Fitted curve is $y = 0.06 + 0.091/x + 0.02/x^2$, $R^2 = 0.14$, $n = 147$, $P < 0.001$. All results plotted are means $\pm$ s.e.m. METHODS. 147 plots, each 3×3 m, with 1-m walkways located at Cedar Creek in Minnesota²⁴, were randomly assigned to one of 7 species-richness treatments: seeding plots in May 1994 to 1, 2, 4, 6 or 8 species (20 replicates each), 12 species (23 replicates), or 24 species (24 replicates). Species used were *Achillea millefolium*, *Agropyron smithii*, *Andropogon gerardi*, *Anemone cylindrica*, *Asclepias tuberosa*, *Aster azureus*, *Astragalus canadensis*, *Bouteloua gracilis*, *Buchloe dactyloides*, *Coreopsis palmata*, *Elymus canadensis*, *Euphorbia corollata*, *Koeleria cristata*, *Lespedeza capitata*, *Liatris aspera*, *Panicum virgatum*, *Petalostemum purpureum*, *Poa pratensis*, *Rudbeckia hirta*, *Schizachyrium scoparium*, *Solidago nemoralis*, *Sorghastrum nutans*, *Sporobolus cryptandrus* and *Vicia villosa*. Species added to each plot were chosen by separate random draws from these 24 prairie perennials. Each plot received 10 g m^{-2} seed in total, with equal masses of each species. To prepare soils for planting, pre-existing vegetation was treated with herbicide and burned in August 1993, the upper 6 to 8 cm of soil removed to reduce the seed bank, then plowed and repeatedly harrowed. Plots were watered twice a week and



were greater under low-diversity treatments (Fig. 1d), indicating greater leaching loss of soil nitrogen at lower plant diversity. Consistent with its low mobility in soil, NH_4^+ showed no such dependence ($r = 0.03$, $n = 147$, $P = 0.7$).

In summary, this experiment shows that both plant productivity and resource utilization were significantly greater at higher plant diversity in these developing grassland ecosystems. The greater nutrient utilization at higher species diversity resulted in lower leaching loss of soil nitrogen, which should contribute to the sustainability of nutrient cycling and soil fertility in these ecosystems.

Native, undisturbed grassland showed similar relationships. Total plant cover increased significantly with plant species richness (Fig. 2a). The most species-rich plots had total cover of about 80%, compared with about 60% in our experiment, indicating that cover may continue to increase in our experiment. However, soil fertility (that is, the total soil nitrogen level, N) was higher in the native grassland than in the experiment ($\text{N}: 686 \text{ mg kg}^{-1}$ compared with 406 mg kg^{-1}), which may explain this difference in plant cover. Rooting-zone extractable soil NO_3^- and NH_4^+ were both negatively correlated with plant-species richness in native grassland (NO_3^- : $r = -0.47$, $n = 120$, $P < 0.001$, Fig. 2b; NH_4^+ :

weeded from an elevated boardwalk. All responses to treatments were measured after two seasons of growth, in August 1995, time of peak standing crop, a measure of productivity in these grasslands. Standing crop was estimated two ways. First, per cent cover of each species was determined in the same, predetermined 0.5×0.5 -m location in all plots for calculation of species richness, total plant cover, H' and $e^{H'}$. Second, light penetration through vegetation, measured 6 times per plot, was converted to biomass using a calibrated relationship. Soil nitrate and ammonium concentrations used 0.01 M KCl extractions, with two cores per plot. Root biomass data in regressions were from cores $5 \text{ cm diameter} \times 20 \text{ cm deep}$. Curves were chosen for simplicity and goodness of fit, and have much higher R^2 values than the corresponding Pearson (linear) correlations (see text).

$r = -0.28$, $n = 120$, $P < 0.01$). As in the experiment (Fig. 1c), native vegetation showed higher variance in soil NO_3^- at lower species richness. Thus the relationships observed in our two-year field experiment also occurred in natural ecosystems, indicating that the effects of biodiversity observed during ecosystem establishment are maintained in mature ecosystems.

Compensatory competitive interactions^{6,7,14} might have played a role in causing these relationships in our experiment. Five species (*Andropogon gerardi*, *Achillea millefolium*, *Bouteloua gracilis*, *Lespedeza capitata*, and *Rudbeckia hirta*) had significantly ($P < 0.01$) greater abundance in higher-diversity plots than expected on the basis of their proportion in the seed mixture, indicating that, when present, they could compensate for poorly performing species. Multiple regression showed that total plant cover in the diversity experiment was negatively dependent on rooting-zone soil NO_3^- and positively dependent on root biomass (Table 1). But there remained a significant dependence of cover on species richness (Table 1), suggesting that additional factors related to species richness also were involved. Other multiple regressions showed that soil NO_3^- , both in the rooting zone (Table 2) and below, was independent of plant cover and surface root biomass, but remained dependent on species richness. Further

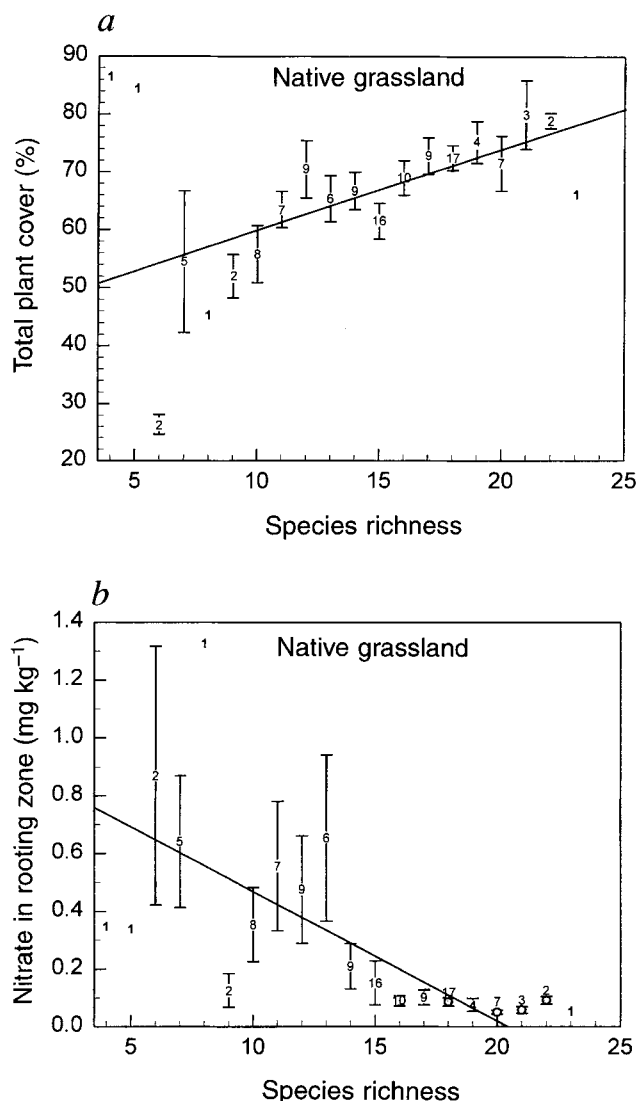


FIG. 2 a, Mean $\pm$ s.e.m. of total plant cover (fitted curve: $y = 46.1 + 1.40x$, $R^2 = 0.16$, $n = 120$, $P < 0.001$) and b, soil nitrate in the rooting zone (fitted curve: $y = 0.92 - 0.045x$, $R^2 = 0.22$, $n = 120$, $P < 0.001$) both plotted against plant-species richness in 120 native grassland plots. Figures indicate the number of plots with a given level of plant species richness. Simple curvilinear fits were no better than lines.

METHODS. Native, undisturbed grassland in Field D (ref. 24) was sampled for plant species cover and species richness (number of vascular plant species per plot) in 120 plots, each 1 m $\times$ 1 m, with a block of 4 such plots in each of 30 localities. Four 0–20 cm soil cores per plot were extracted for measuring NO_3^- and NH_4^+ levels.

work is needed to determine how interspecific morphological and physiological differences^{25–27} influence the dependence of ecosystem functioning on biodiversity in this and other ecosystems.

It is known that soil fertility and productivity influence diversity^{28,29}. Our results demonstrate that the converse is also true: in our experiment using initially homogeneous soils, plant diversity had a significant effect on productivity, nutrient use, and nutrient retention. The establishment and functioning of these grassland ecosystems depended on their species richness, with more diverse ecosystems being more productive and having lower nutrient losses than less diverse ecosystems. This extends earlier results^{8,18} to the field, providing direct evidence that the current rapid loss of species on Earth³⁰, and management practices that decrease local biodiversity, threaten ecosystem productivity and the sustainability of nutrient cycling. Observational, laboratory and now field experimental evidence supports the hypotheses that bio-

diversity influences ecosystem productivity^{6,8,17,18}, sustainability^{4,5,17} and stability^{3,6,7,14}. □

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Allometry and simple epidemic models for microparasites

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SIMPLE mathematical models for microparasites offer a useful way to examine the population dynamics of different viral and bacterial pathogens. One constraint in applying these models in free-living host populations is the paucity of data with which to estimate transmission rates. Here we recast a standard epidemiological model by setting the birth and death rates of the host population and its density as simple allometric functions of host body weight. We then use standard threshold theorems for the model in order to estimate the minimum rate of transmission for the parasite to establish itself in a mammalian host population. Transmission rates that produce different comparable values of the parasites' basic reproductive number, R_0 , are themselves allometric functions of host body size. We have extended the model to show that hosts having different body sizes suffer epidemic outbreaks whose frequency scales with body size. The expected epidemic periods for pathogens in different mammalian populations correspond to cycles observed in free-living populations.

The basic microparasite model takes the form¹ $dS/dt = (\nu - \mu)(1 - S/K)S - \lambda(I)S$, and $dI/dt = \lambda(I)S - (\mu + \alpha)I$, where S and I are the density of susceptible and infected individuals respectively, K the carrying capacity in absence of the pathogen^{2,3},

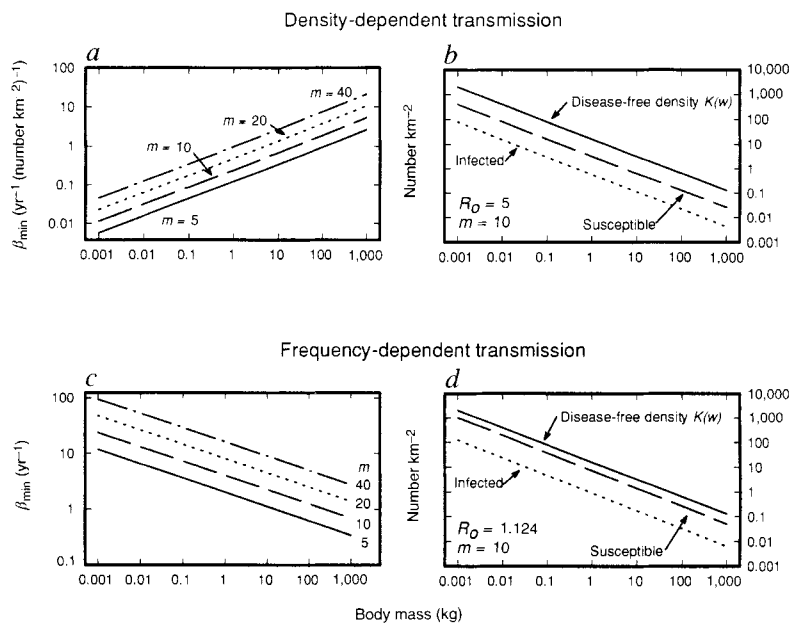
FIG. 1 Allometric relationships of $\beta_{\min}$ for density-dependent transmission (in a, $\beta_{\min} = 0.0247 m w^{0.44}$) and frequency-dependent transmission (in c, $\beta_{\min} = 0.4 m w^{-0.26}$). Host demographic parameters are: $K = 16.2 w^{-0.70}$ (number km^{-2}), $r = v - \mu = 0.6 w^{-0.27}$ (years $^{-1}$), $\mu = 0.4 w^{-0.26}$ (years $^{-1}$); m indicates the disease-induced mortality factor for infected individuals. If infection spreads ($\beta > \beta_{\min}$), host density will drop below the natural carrying capacity. Densities of susceptibles and infectives at equilibrium are inversely related to w (b and d). In contrast, the fraction of infected hosts and the degree of depression, $d = N_{\text{eq}}(w)/K(w)$, below the carrying capacity are independent of w . To derive the relationships in b and d, transmission rates have been chosen to produce comparable values of R_0 for mammals of different size, namely $\beta(w) = 5\beta_{\min}(w)$, and thus $R_0 = K\beta/(\alpha + \mu) = 5$ in the case of density-dependent transmission, and $\beta(w) = 1.124\beta_{\min}(w)$, and thus $R_0 = \beta/(\alpha + \mu) = 1.124$ in the case of frequency-dependent transmission. In the latter case, the host population is always driven to extinction when $R_0 \geq 1.22$. The allometric exponents of $I_{\text{eq}}(w)$ and $S_{\text{eq}}(w)$ are, to a large extent, determined by the carrying capacity $K(w)$ of the disease-free population, and are largely insensitive to the actual values of R_0 and α (provided $R_0 > 1$). Although transmission parameters combine several different epidemiological, environmental and social factors, the scaling of $\beta_{\min}$ is basically due to the following reasons: $\beta_{\min}$ increases with w in the density-dependent case because the number of encounters per unit time decreases with body size as a consequence of the lower population density, thus higher transmission rates are required for the

μ the host natural mortality rate, v the maximum birth rate, α the disease-induced mortality, and $\lambda(I)$ the 'force of infection', that is, the rate at which animals become infected. The actual relationship between λ and I depends upon the type of interaction among infected and susceptibles. We examine two main types of transmission: density-dependent, in which the probability of a susceptible becoming infected is proportional to the density of infectives through the transmission rate β , namely $\lambda(I) = \beta I$; and frequency-dependent, in which the same probability is a function of the proportion of infectives, namely $\lambda(I) = \beta I/N$ (where $N = I + S$). Density-dependent transmission is usually assumed for wild-animal diseases^{4,5}, but frequency dependence may characterize sexually and vectorially transmitted pathogens^{6,7}. It may also apply to species that are strongly territorial.

Following the introduction of a single infective into a population of K susceptibles, the disease will only spread provided $dI/dt > 0$. For this to occur, β must exceed a critical value $\beta_{\min}$, where $\beta_{\min} = (\mu + \alpha)/K$ for density-dependent transmission, and $\beta_{\min} = \mu + \alpha$ for frequency-dependent transmission. These thresholds are directly related to the basic reproductive number R_0 , defined as the average number of secondary cases generated by one primary case in a susceptible population^{5,8}. The disease spreads in the population if $R_0 \geq 1$.

These threshold theorems are very useful in investigating disease dynamics and control policies like culling and vaccination, but their use is hampered in practice because direct measurements of β are difficult, if not impossible, to obtain without extensive field data^{1,9}. In contrast, estimates of the basic host demography (v , μ and K) are available for many species. Extensive comparative studies¹⁰⁻¹⁴ relate v , μ and K to body size and show that they scale with height w as simple allometric relationships.

We can thus derive a similar allometric relationship for the transmission rate and predict the threshold values of β for species over a wide range of body sizes. To generalize this calculation we rescale the disease-



disease to spread. In contrast, when transmission is frequency-dependent, the probability of successfully transmitting the disease does not depend upon density, but is proportional to the time spent in the infective class, $(\alpha + \mu)^{-1}$; as larger individuals have a longer infectious period, $\beta_{\min}$ decreases with w .

induced mortality rate α with respect to the average lifespan of the species. By assuming that α is $(m - 1)$ times the natural mortality μ , more (or less) virulent pathogens will be characterized by larger (or smaller) values of m , as this produces an m -fold reduction of the $(\mu + \alpha)^{-1}$ life expectancy of infected individuals.

After replacing $v(w)$, $\mu(w)$, $K(w)$ and $\alpha(w)$ into the equations for

FIG. 2 The oscillation period T (in years) of the host population as a function of the body mass w (in a), the basic reproductive number $R_0 = K\beta/(\alpha + \mu)$ (in b) and the disease-induced mortality factor m (in c) in the case of density-dependent transmission. Note that for a pathogen that produces a 5–20-fold reduction of life expectancy of a 1-kg animal, the oscillation period ranges between 3 and 7 years, whereas T is practically constant for $R_0 > 3$. Scaling of the oscillation period is basically determined by the scaling of the intrinsic growth rate¹⁶. The stability properties and the transient dynamics to equilibrium are different when transmission is frequency-dependent: when $w < 10$ kg, the system approaches equilibrium without oscillations, whereas for $w > 10$ kg, damped oscillations with a very long period (> 60 years) may occur. In any case, no significant relationship can be detected between body size and equilibrium eigenvalues.

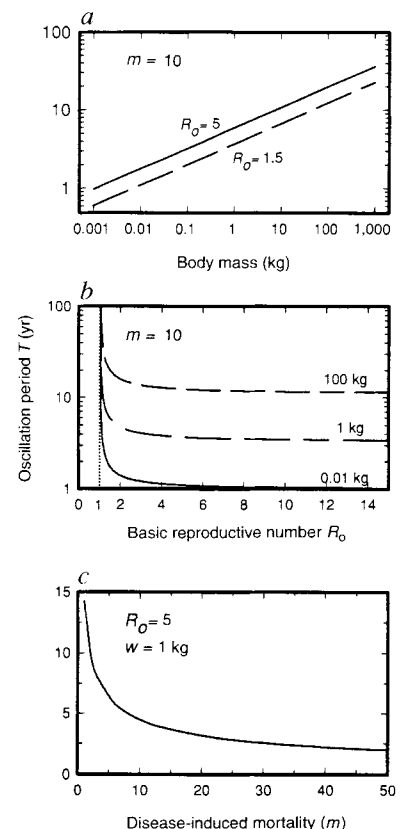


TABLE 1 Threshold conditions on β for five epidemiological models assuming different courses of infection and the density dependence of the host

Model	Basic reproductive number	Threshold criterion for transmission rate	Allometric relationship $\beta(w)$
a, SI model with nonlinear density-dependent fecundity $S' = (v - \mu)[1 - (N/K)^c]S - \beta SI$ $N = I + S$ $c > 1$ $I' = \beta SI - (\alpha + \mu)I$ $\alpha = (m - 1)\mu$	$R_0 = \frac{K\beta}{\alpha + \mu}$	$\beta \geq \beta_{\min} = \frac{\alpha + \mu}{K}$	$\beta_{\min} = 2.47 \cdot 10^{-2} m w^{0.44}$
b, SI model with density-dependent adult mortality $S' = (v - \mu)[1 - N/K]S - \beta SI$ $N = I + S$ $I' = \beta SI - (\alpha + \mu + \gamma N)I$ $\gamma = (v - \mu)/K$	$R_0 = \frac{K\beta}{\alpha + v}$	$\beta \geq \beta_{\min} = \frac{\alpha + v}{K}$	$\beta_{\min} = (2.47 m + 8.02) \cdot 10^{-2} w^{0.44}$
c, SIR: susceptible, infective and recovered $S' = (v - \mu)[1 - F/K]S - \beta SI$ $F = S + R$ $I' = \beta SI - (\alpha + \mu + \rho)I$ $R' = \rho I - \mu R$	$R_0 = \frac{K\beta}{(\alpha + \mu + \rho)}$	$\beta \geq \beta_{\min} = \frac{\alpha + \mu + \rho}{K}$	$\beta_{\min} = 2.47 \cdot 10^{-2} (m + n) w^{0.44}$ where $n \equiv \rho/\mu$
d, SEI: susceptible, exposed and infective $S' = (v - \mu)[1 - F/K]S - \beta SI$ $F = S + E + I$ $E' = \beta SI - (\sigma + \mu)E$ $I' = \sigma E - (\alpha + \mu)I$	$R_0 = \frac{\sigma\beta K}{(\sigma + \mu)(\alpha + \mu)}$	$\beta \geq \beta_{\min} = \left(1 + \frac{\mu}{\sigma}\right) \frac{\alpha + \mu}{K}$	$\beta_{\min} \approx 2.47 \cdot 10^{-2} m w^{0.44}$ when $\sigma \gg \mu$
e, SEIR: susceptible, exposed, infective and recovered $S' = (v - \mu)[1 - F/K]S - \beta SI$ $F = S + E + R$ $E' = \beta SI - (\sigma + \mu)E$ $I' = \sigma E - (\alpha + \mu + \rho)I$ $R' = \rho I - \mu R$	$R_0 = \frac{\sigma\beta K}{(\sigma + \mu)(\alpha + \mu + \rho)}$	$\beta \geq \beta_{\min} = \left(1 + \frac{\mu}{\sigma}\right) \times \frac{\alpha + \mu + \rho}{K}$	$\beta_{\min} \approx 2.47 \cdot 10^{-2} (m + n) w^{0.44}$ where $n \equiv \rho/\mu$ and $\sigma \gg \mu$

v and μ are the maximum fecundity and minimum mortality, respectively. The allometric relationship $\beta(w)$ between transmission and body size does not change with respect to the basic SI model when infected individuals can reproduce and the density-dependent reduction in host fecundity is not linear (model a). If density affects adult mortality rather than fecundity (model b), the threshold condition becomes $\beta \geq \beta_{\min} = (v + \alpha)/K$. As fecundity has roughly the same allometric coefficient as that of host mortality, $\beta_{\min}$ scales with body mass as in model a. Furthermore, if the disease-induced mortality factor m equals 20, $\beta_{\min}$ is only about 10% larger than in model a for the same m . The introduction of an immune class (model c) or a latent class (models d and e) slightly complicates the formula for the β threshold, but does not modify the allometry of $\beta_{\min}$ with respect to the previous models. In fact, the average time spent in the latent class, $1/\sigma$, is usually much smaller than the average life expectancy $1/\mu$, therefore $\mu/\sigma \approx 0$. Also, the rate of recovery ρ for the immune class can reasonably be assumed to scale with body size as μ . This explains why the exponent of the w - β relationship is the same as usual. Although the allometric property of the threshold criterion is basically insensitive to variation in the model structure, the actual dynamics may change considerably as models with a latent class may show complex dynamics (such as limit cycles and chaos) if the basic reproductive number is sufficiently high, whereas the simple SI model cannot, unless exogenous seasonal factors are explicitly introduced.

$\beta_{\min}$, we find that it scales allometrically with body size. $\beta_{\min}$ increases with w when transmission is density-dependent and decreases when transmission is frequency-dependent (Fig. 1). With density-dependent transmission, the model exhibits damped oscillations towards equilibrium densities for any body size (Fig. 1), provided $\beta(w) > \beta_{\min}(w)$. The oscillation period is allometric (Fig. 2), with an exponent of 0.260. Peterson *et al.*¹⁵ analysed population cycles of 41 species of birds and mammals: they found that populations fluctuate with a period related to body mass as $8.51w^{-0.263}$. Although our finding matches their data very well, other factors such as spatial and age structure, predation, plant-herbivore relationships and seasonality may be important in explaining the observed patterns. Oscillations are not detectable in the frequency-dependent case, which actually applies only to a minority of wild-animal diseases.

This model does not incorporate many features of the population at the epizootological level, such as different forms of density-dependent growth and the presence of latent and/or recovered classes. But several logical embellishments do not significantly modify the relationship between the body size of the host and the characteristic transmission rate: allometry holds for a wide class of epidemiological models (Table 1). These relations should not be interpreted as deterministic laws giving the exact transmission rate for any species, however, because other important details must be considered. For instance, there is considerable variability in the relationship between population density and body size¹⁴. Several studies^{10,13} have shown that carnivore populations are significantly less dense than those of herbivores and frugivores, so the relationships we described should be modified to include these differences. Also, the two components of the transmission rate should be included: production of infective agents and actual transmission of those agents from infective to susceptible. Large body size

may allow greater production of infective viral particles—this would boost the transmission rate in the absence of any compensatory decrease in the actual transmission rate of pathogens.

The allometric relationships shown in Table 1 are a good indication of the general trends underlying the available data and provide a useful initial estimate of β for pathogens of mammals over a wide range of body sizes and population densities. The results in Fig. 1a,c offer an important insight into whether emergent diseases such as Hantavirus and Ebola virus could become established in the human population. If pathogens use mammals as their normal hosts and transmission is density-dependent, then outbreaks of these pathogens should die out in all but the most crowded human populations; in contrast, if transmission is frequency-dependent, as in the case of human immunodeficiency virus, they should be able to become established. □

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Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production

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THEORIES of life-history evolution propose that trade-offs occur between fitness components, including longevity and maximal reproduction¹⁻³. In *Drosophila*, female lifespan is shortened by increased egg production⁴, receipt of male accessory fluid⁵ and courting⁶. Male lifespan is also reduced by courting and/or mating⁷. Here we show that in the nematode *Caenorhabditis elegans*, mating with males reduces the lifespan of hermaphrodites by a mechanism independent of egg production or receipt of sperm. Conversely, males appear unaffected by mating. Thus, in *C. elegans* there is no apparent trade-off between longevity and increased egg or sperm production, but there is a substantial cost to hermaphrodites associated with copulation.

The soil-dwelling roundworm *C. elegans* can reproduce either as a self-fertilizing hermaphrodite or by crossing with males. Hermaphrodite reproduction is limited by the number of sperm produced (around 300) because the adult switches to production of oocytes. Oocyte production ceases after depletion of sperm. Cross-fertilization can increase brood size to over 1,000 (ref. 8). Hermaphrodite reproduction *per se* has no detectable effect on longevity since wild-type lifespans have been observed in sterile mutants⁹⁻¹¹ and in animals in which the gonadal precursor cells had been destroyed by laser microsurgery¹¹. We tested the effect of exposure to males on lifespan. Using the wild-type (N2) strain and conditions similar to a previous study (ref. 12; Fig. 1), we observed that hermaphrodite lifespan (50% survival) was reduced by 43%, from 16.7 ± 0.5 to 9.6 ± 0.5 days (Fig. 1a). Furthermore, hermaphrodite longevity was found to vary with the dosage of males (Fig. 1c and Table 1). The increase in mortality reached saturation at a male-to-hermaphrodite ratio of 1.5–2.0 to 1, where a 50% reduction in lifespan was seen. Below this ratio, crossing was less than 100% efficient (that is, there were fewer than 50% male progeny). Exposure to hermaphrodites did not affect male survival, even at low male-to-hermaphrodite ratios (Fig. 1b and Table 1).

Our results are the opposite to those reported by Van Voorhies¹², who observed a reduction of lifespan in mated *C.*

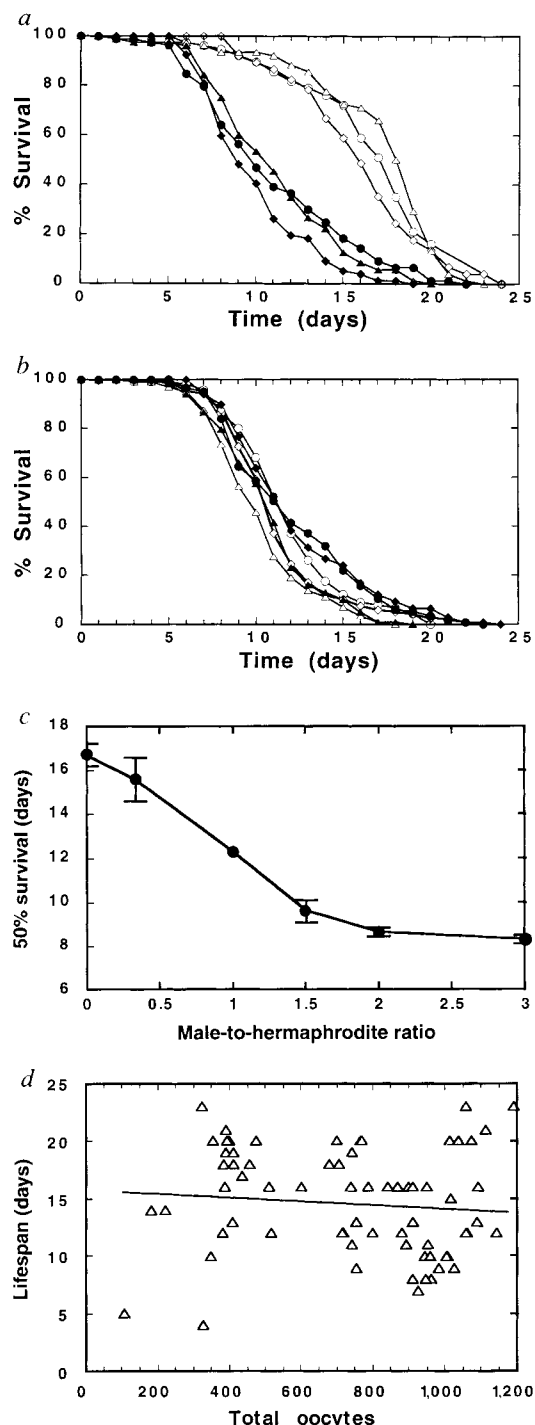


FIG. 1 Effect of mating on *C. elegans* wild-type males and hermaphrodites. a, Hermaphrodites; b, males. Fifteen L4 males and ten L4 hermaphrodites were placed on each plate as previously described¹². Total sample size: 229 unmated, 236 mated hermaphrodites; 379 unmated, 358 mated males. Filled symbols, mated; open symbols, unmated. The three different symbols represent three replicate experiments. Hermaphrodite lifespan (50% survival) was significantly decreased by mating (Mann Whitney $U = 0.00$, d.f. = 1, $P < 0.05$). Conversely, male lifespan (50% survival) was unaffected ($U = 7.00$, d.f. = 1, $P > 0.05$). In two replicates, 60-mm Petri plates were used, and in the third, 35-mm plates were used as described¹². Under these conditions it was also found that incomplete cross-fertilization occurred (60–95%, measured as twice the percentage of male offspring). The small subset of hermaphrodites in the mating experiment that survived to the same maximum lifespan probably experienced partial or no cross-fertilization. The degree of crossing varies with parental sex ratio. c, Effects of male dosage on hermaphrodite survival. Error bars represent standard error. d, Brood sizes and lifespans of individual briefly mated hermaphrodites.

METHODS. Animals were maintained monoxenically at 20 °C in 60-mm Petri dishes filled with NG agar and seeded with *E. coli* OP50 as the food source²³. To determine adult lifespans, 15–200 L4 larvae were transferred to fresh plates, then transferred again daily during the egg-laying period,

and less frequently thereafter. Death was scored as absence of any movement and failure to move after several light pokes with a platinum wire. The zero point was the time of L4 transfer. To minimize possible costs of copulation and achieve the broad range of brood sizes shown in d, each hermaphrodite was mated with three males on a 1-cm spot of bacteria for 5 h at 20 °C, after which the males were removed. Owing to the brevity of the mating period, brood size should, in most cases, be limited by the number of sperm transferred, and not the fitness of the individual hermaphrodites. Animals were transferred daily to fresh plates and each day's progeny counted two days later. Of 62 hermaphrodites incubated with males, 49 proved to have mated on the basis of the presence of male offspring. A further eight unmated hermaphrodites were also scored. A total of 52,086 progeny (48,350 progeny and 3,736 unfertilized oocytes) were scored. The curve was generated by the bestfit function of KaleidaGraph version 2.1.3 (Abelbeck Software). Lifespan was not correlated with brood size ($r = 0.10$, $P \gg 0.05$). Conclusions were the same when fertilized oocytes were excluded.

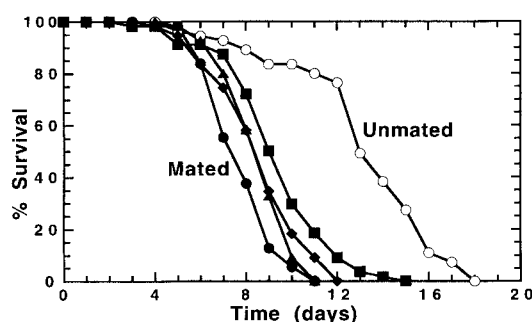


FIG. 2 Lifespan (50% survival) of N2 hermaphrodites at 25 °C was shortened by mating, even with fertilization-defective males (mated versus unmated; $t = 6.70$, $P < 0.05$). Results shown are from one of two duplicate experiments that yielded similar results. The male-to-hermaphrodite ratio was 2:1. The mean sample size per curve was 54.8. As prior tests showed that *fer-1* and *fer-6* males had a reduced lifespan relative to N2 males (50% survival: 5.6, 5.9 and 8.5 days, respectively), males were replaced every 48 h. Survival of hermaphrodites mated with an unreplenished population of N2 males is also shown. Open circles, unmated; diamonds, unreplenished N2 male-mated; filled circles, N2 male-mated; triangles, *fer-1* male-mated; squares, *fer-6* male-mated. Note: the *fer-6(hc6ts)* strain used, BA6, also contains a weak *unc* (uncoordinated) mutation²⁴.

elegans males but not hermaphrodites. Van Voorhies used a short-lived variant of N2 with a hermaphrodite lifespan (50% survival) of 12.0 ± 0.3 days (20 °C). The N2 strain used in our study was the *Caenorhabditis* Genetics Center (CGC) reference N2 male stock. F1 hermaphrodites from crosses between the CGC N2 strain and Van Voorhies' variant had a lifespan indistinguishable from the CGC strain, indicating that the Van Voorhies variant is a recessive, short-lived mutant. However, strain differences fail to explain why our more extensive study differs from the previously reported results, for in our hands the short-lived variant also showed hermaphrodite- but not male-mating costs when grown on the standard food source, *E. coli* strain OP50 (data not shown).

There are several possible causes of reduced lifespan in hermaphrodites exposed to males: (1) metabolic costs of extra egg production; (2) mating-induced physiological changes associated with increased rate of egg production; (3) receipt and/or storage of sperm or seminal fluid; (4) internal hatching of larvae; (5) structural damage sustained during copulation; (6) mating-associated bacterial infection; and (7) non-mating costs associated with the presence of males.

Possible costs of increased egg production were assayed by briefly mating young adult hermaphrodites and comparing brood size with lifespan in individual animals (Fig. 1d). Only a small number of unfertilized oocytes are stored by the hermaphrodite, so increased broods require increased investment of resources in oocyte production. No significant correlation between brood size and lifespan was observed, from which we conclude that the decrease in lifespan is not the result of increased egg production. Another possibility is that mating stimulates a physiological response in hermaphrodites associated with resource mobilization to increase the rate of egg production at a cost to somatic maintenance. However, we did not observe an increase in the rate of egg production associated with mating (Table 2).

To investigate whether receipt or storage of sperm affect hermaphrodite lifespan, N2 hermaphrodites were mated with

sterile males. Mutant *fer-1(hc24ts)* males mate normally at 25 °C and transfer sperm that are capable of stimulating oocyte production but incapable of fertilization^{13,14}, whereas *fer-6(hc6ts)* males appear to copulate normally, but neither stimulate oocyte production nor transfer sperm^{13,15}. Figure 2 shows that hermaphrodite lifespan is decreased by mating with either *fer-1* or *fer-6* males. As *fer-6* males do not transfer sperm, this indicates that neither receipt nor storage of sperm is the cause of hermaphrodite lifespan reduction. In *Drosophila*, seminal fluid has been shown to stimulate egg production, suppress fertilization by sperm from previous matings, and decrease female lifespan^{5,16-19}. If the cost of mating with *fer-6* males were a side effect of displacement of self-sperm caused by seminal fluid, it might be expected that mating N2 hermaphrodites with *fer-6* males would also result in a reduction of egg laying due to inhibition of self-fertilization, but this was not seen (data not shown). However, it remains possible that *fer-6* males transfer seminal fluid, and that this reduces hermaphrodite lifespan.

We tested whether hatching of larvae inside the uterus caused early hermaphrodite mortality by mating sterile hermaphrodites with sterile males. Mutant *fog-2(q71)* hermaphrodites produce no sperm²⁰, so mating with *fer-6(hc6ts)* males at 25 °C produces very few or no fertilized eggs. In two trials using *fer-6* males at a male-to-hermaphrodite ratio of 2:1, a hermaphrodite lifespan of 7.8 ± 0.8 days (25% survival) was seen, compared to 6.5 ± 0.7 days when N2 males were used, and 14.1 ± 0.8 days for unmated hermaphrodites. (Results are expressed as 25% survival because a proportion of unmated *fog-2* hermaphrodites die as young adults.) This result indicates that internal hatching of larvae is not the cause of reduced hermaphrodite lifespan, and it provides further evidence that reduced lifespan is not the result of increased egg production.

To test whether the mere presence of males decreased hermaphrodite longevity, *mab-10(e1248);him-5(e1490)* males, which cannot copulate because of tail defects²¹, and *unc-32(e189);him-8(e1489)*

TABLE 1 Effect of sex ratio on longevity (20 °C)

Males/ hermaphrodites	50% Survival of males (days $\pm$ s.e.)	50% Survival of hermaphrodites (days $\pm$ s.e.)	Maximum lifespan of males (days $\pm$ s.e.)	Maximum lifespan of hermaphrodites (days $\pm$ s.e.)	N*	Total hermaphrodites
0	—	16.7 ± 0.5	—	23.6 ± 0.6	9	513
0.33	12.2	15.6 ± 1.0	24	23 ± 1.0	2†	148
1.0	11.8	12.3	21	20	1	50
1.5	10.9 ± 0.2	9.6 ± 0.5	21 ± 1.5	19.3 ± 0.9	3	224
2.0	9.5 ± 0.4	8.6 ± 0.2	21 ± 1.5	14.8 ± 1.2	5	251
3.0	11.9 ± 0.4	8.3 ± 0.2	22.3 ± 2.2	13.5 ± 0.4	3	103
—	10.1 ± 0.2	—	18.1 ± 1.2	—	9	—

Male: hermaphrodite ratio (M:H) explains 86.5% of the variation in 50% survival of hermaphrodites (Pearson's $r = 0.93$; all statistics estimated with SYSTAT²²). The sex ratio effect appeared saturated when $M:H \geq 1.5$ ($r = -0.61$, $P = 0.06$). Male 50% survival was not correlated with M:H ratio ($r = 0.11$, $P \gg 0.05$).

* Number of independent trials. Mean sample size was 64.7 (range: 31–162). Lifespans were found to be independent of population density at the densities used. Animals lost during trials (on average 6.4 and 8.6% of the initial number of hermaphrodites and males, respectively) are excluded from these values. Note that mating causes a slight increase in male maximum lifespan.

† Values for males from two trials were summed to yield an adequate sample size. This was treated as a single trial for males.

TABLE 2 Effect of mating on egg production rate

Treatment	Eggs laid per hour		
	Day 1	Day 2	Day 3
Unmated	6.1 ± 0.2	6.5 ± 1.5	0.6 ± 0.3
Mated once	5.0 ± 0.2	6.9 ± 0.2	5.4 ± 1.9
Mated thrice	4.9 ± 0.4	6.5 ± 0.1	5.1 ± 1.3

N2 hermaphrodites were mated singly with 10 N2 males for five hours (20 °C). The first group was unmated, the second mated only on day one, and the third on three consecutive days. Egg laying was measured over a three-hour period at 24-hour intervals, immediately after mating in the mating test groups. Each group consisted of six animals and the entire experiment was repeated twice. Rates are $\pm$ s.e. Non-parametric ANOVA (Kruskal-Wallis) revealed a nearly significant decrease in the rate of egg laying in the two mated groups on day 1 ($P = 0.07$), but not day 2. No increase in egg-laying rate was observed. On day 3, the self sperm of unmated animals were almost depleted.

males, which cannot mate owing to motility defects²¹, were used. Maintenance in the presence of such males throughout life, at a male-to-hermaphrodite ratio of 2:1, did not reduce hermaphrodite longevity (data not shown).

These results demonstrate that the cost of mating to hermaphrodite longevity is not associated with egg production or egg laying, the receipt or storage of sperm, internal hatching of eggs, or non-mating effects associated with presence of males. Thus, it would appear that the stress of copulation reduces hermaphrodite lifespan, either directly or by increasing susceptibility to infection by the bacteria used as food. It also remains possible that receipt of seminal fluid is a cause. By contrast with the hermaphrodites, there is no apparent cost to males of increased spermatogenesis or copulation. Sterile hermaphrodites do not have increased lifespan⁹⁻¹¹ and mutations in only one of many genes involved in spermatogenesis, *spe-26*, have been reported to increase lifespan¹², suggesting that lifespan extension in *spe-26* mutants may be a pleiotropic effect unconnected to the defect in spermatogenesis. Taken together, these results do not support the hypothesis that increased gamete production accelerates ageing because of a drain on resources from somatic maintenance functions, or that mating actively stimulates such a diversion of resources. It is possible, however, that such trade-offs might only be observed when resources are limited, that is, in calorically restricted animals. □

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A systematic map of direction preference in primary visual cortex

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NEURONS in the primary visual cortex respond selectively to the orientation of edges and their direction of motion. Orientation preference is mapped in a systematic fashion across the cortical surface, such that neurons in adjacent columns have similar but slightly shifted preferred orientations¹⁻⁷. Microelectrode studies have suggested that direction preference is also arranged in a systematic fashion⁸⁻¹⁰, but exactly how this response property is mapped remains unclear. Here we show by optical imaging⁴⁻⁵ of intrinsic signals^{6-7,11-14} in ferret cortical area 17 that there is a mosaic-like map of direction preference. This map consists of numerous regions within which direction preference changes in a slow, continuous fashion. These regions are separated by winding boundaries (fractures) across which direction preference shifts abruptly, often by 180°. Comparison of direction and orientation preference maps shows that these fractures subdivide iso-orientation domains into regions selective for opposite directions of motion.

Functional maps of direction preference were obtained by collecting separate images of the cortical surface while the animal viewed bar stimuli moving in one of eight directions ($n = 13$ animals). Figure 1a shows differential images of direction preference obtained by subtracting the cortical images that were collected while the animal viewed stimuli that moved in opposite directions. These difference images are composed of numerous oval or circular-shaped dark and light patches (iso-direction domains). Iso-direction domains differ in their appearance from iso-orientation domains, which have a more banded or striped appearance (Fig. 1b). Neurons located within the centre of iso-direction domains have highly selective directional responses that can be predicted from the optical images ($n = 4$ animals, 10 recordings) (Figs 1b, c and 2a).

The complete organization of direction preferences across a region of visual cortex was determined by vectorially summing the eight images shown in Fig. 1a. The result is shown in Fig. 2a, where preferred direction is colour coded and indicated by the direction of the arrows overlaid on the map; the length of the arrows represents the magnitude of direction selectivity. The map consists of numerous, distinct regions of high selectivity in which direction preference shifts in a slow, continuous fashion. Figure 2b shows that these regions are separated by narrow bands of low selectivity across which direction preference changes abruptly, often by 180°. In this image, arrows indicating direction preference are overlaid on a magnitude map of direction selectivity where black indicates regions of low selectivity.

Direction and orientation preference maps are similar in some characteristics and strikingly different in others. Both maps are organized at a similar spatial scale (Fig. 3a, d). Using autocorrelation analysis, we calculated the average repeat distance in the orientation preference map to be $890 \pm 29 \mu\text{m}$ and in the direction preference map to be $867 \pm 18 \mu\text{m}$ ($n = 5$ animals). But the fragmented structure of the direction preference maps, which is especially apparent when the magnitude of the direction signal is plotted (Fig. 3b), is very different from that found for orientation preference maps (Fig. 3e). Regions of low selectivity in orientation maps are confined to small circular zones located at the centres of orientation pinwheels or, less commonly, short lines that connect adjacent pinwheel centres⁵⁻⁷. This difference is also apparent

when rate of change maps for direction and orientation preference are compared (Fig. 3c, f); regions of low selectivity in both maps correspond to the regions in which the mapped response property changes most rapidly. Thus orientation maps are dominated by points of rapid change whereas direction maps are dominated by lines of rapid change (fractures).

The fragmented structure of the direction preference map may be understood in terms of the need to represent all orientations and directions for every point in visual space. To satisfy this requirement, two opposite directions of motion must be represented for each stimulus orientation. Consistent with this interpretation, we found that single iso-orientation domains are subdivided into numerous patches selective for motion in opposite directions (Fig. 4a). This subdivision of iso-orientation regions is also apparent when vector maps of direction and orientation preference are overlaid (Fig. 4b) and when direction fractures are viewed in relation to iso-orientation contour lines (Fig. 4c).

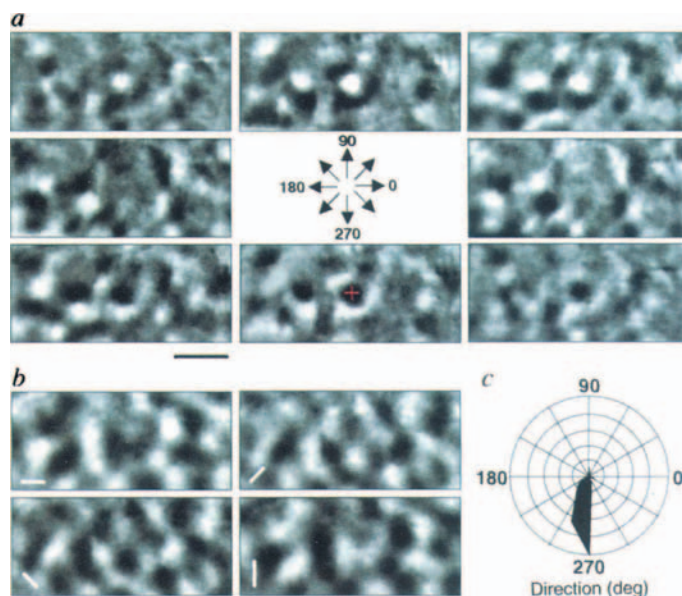
Even though the direction preference map is more fractured than the orientation map, both maps display a high degree of continuity. Indeed, the number and arrangement of direction fractures appears to maximize continuity in the mapping of direction preference within the constraints imposed by the orderly map of orientation. This is best illustrated by examining the

direction preference map in the region of an orientation pinwheel. An orientation pinwheel represents a complete and continuous cycle of orientation values (each orientation appearing only once^{6,7}). Although a complete cycle of orientation values is covered in 180° , the complete cycle of direction values requires 360° (that is, for each orientation there are two possible directions). Thus, even if direction is mapped as continuously as possible around an orientation pinwheel, at most, only half a direction cycle can be represented, and there must be at least one discontinuity in the direction preference map where contiguous cortical regions have opposite direction preferences. That we find examples of orientation pinwheels with single direction discontinuities (Fig. 4d) indicates that continuity is an important feature of the direction preference map. Continuity in the orientation map, however, seems to take precedence over continuity in the direction map. In principle, continuity in both maps could be equally preserved if there were 360° , not 180° pinwheels where each orientation appeared twice^{15,16}, but such 360° pinwheels have not been observed in the visual cortex of any species^{4,7,12,13,17}.

In conclusion, we have demonstrated that direction preference, like orientation preference, is systematically mapped in primary visual cortex. Our results are consistent with the evidence from closely spaced microelectrode recordings suggesting an ordered

FIG. 1 Optical imaging of iso-direction and iso-orientation domains in area 17 of ferret visual cortex. **a**, Differential images of iso-direction domains produced by subtracting images obtained from oppositely moving stimuli. The horizontal arrow pointing to the right corresponds to 0° , whereas 90° is up. The dark regions were strongly activated by movement in the direction indicated by the arrows; light regions were strongly activated by movement in the opposite direction. Iso-direction domains typically appeared as circular or elongated regions of cortical activation, where domains having opposite direction preference were often paired. Scale bar, 1 mm; applies to **a** and **b**. **b**, Differential images of iso-orientation domains. The animal viewed the same stimuli used in **a** but moving in both directions during each presentation. These maps were produced by subtracting images obtained from orthogonal stimuli. Dark regions were strongly activated by the orientation shown in the lower left corner of each panel. In general, orientation domains had a more banded or striped appearance, and were typically larger in size and more uniform in their distribution than direction domains. **c**, Normalized polar diagram showing response versus stimulus direction for a group of neurons recorded at the site marked by the red cross in the lower middle panel in **a**. The neurons were strongly selective for a horizontal elongated bar moving downward (270°), and were located within the centre of an isodirection domain.

METHODS. Experiments were conducted using 13 animals with similar results. Optical imaging was performed as previously described^{12,13}. Images were acquired using an enhanced video acquisition system (Optical Imaging Inc.). Ferrets (postnatal day 43–60) were anaesthetized with a mixture of ketamine hydrochloride (40 mg per kg intramuscularly (i.m.)) and xylazine hydrochloride (2 mg per kg i.m.). A hole (6 mm $\times$ 12 mm) was made in the cranium over area 17, the dura was removed, and a stainless steel chamber was mounted to the skull, filled with saline, and sealed with a coverglass. Ferrets were paralysed with pancuronium bromide (2–4 mg kg⁻¹ interperitoneally) to prevent eye movements and artificially respiration with a mixture of 2:1 N₂O:O₂ supplemented with 0.5–1.0% halothane. Expired CO₂ was maintained at 4–5%. Visual stimulation was provided monocularly through the contralateral eye. The stimulus consisted of an array of randomly placed high-contrast bars ($1.2^\circ \times 4.2^\circ$) that was drifted by $12\text{--}20\text{ deg s}^{-1}$. The average density of bars was 2.5 bars per $10^\circ \times 10^\circ$ region. The orientation of the bars always remained perpendicular to the direction of motion. The stimulus was shown first for 1 s before each period of recording video images. Images were then acquired for 3–15 s during continued stimulus presentation. Direction and orientation preference maps were acquired during separate interleaved sessions. In direction preference mapping sessions, the stimulus was moved in one direction throughout each acquisition period. Stimuli were paired so that an acquisition period with the stimulus moving in one direction was followed by an 8-s interstimulus interval (screen blank) and



then another acquisition period during which the stimulus moved in the opposite direction. Multiple direction mapping sessions for each of four pairs of directions were conducted. In orientation selectivity mapping sessions the stimulus was drifted back and forth during each acquisition period, and stimuli were paired so that an acquisition period with the stimulus at one orientation was followed by an 8-s interstimulus interval and then an acquisition period with the stimulus at the orthogonal orientation. Multiple orientation mapping sessions for each of two pairs of orientations were conducted. In direction and orientation mapping sessions, 15–30 stimulus pairs were presented and all data acquired for each orientation and direction were averaged. We confirmed that this method of paired stimulus presentation produced the same results as when both orientation and direction maps were derived from a single set of acquired images (a single experiment with random presentation of 8 single-direction stimuli). Stimulus pairing, however, consistently produced images with a better signal-to-noise ratio. Images were acquired at a resolution of 655 by 480 pixels which corresponds to $13\text{ }\mu\text{m}$ per pixel with our camera optics. All differential images were smoothed with kernels ranging from 5–13 pixels, to reduce noise.

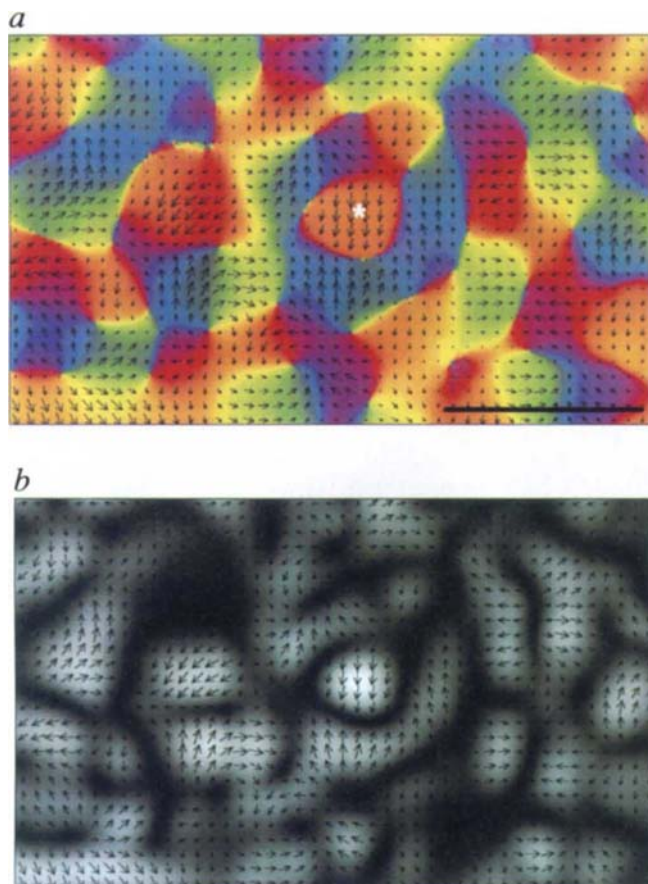
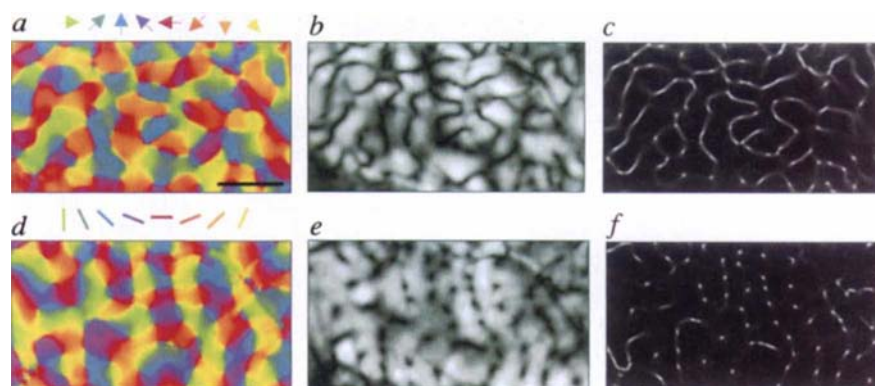


FIG. 2 Map of direction preference, produced by vectorially summing the images shown in Fig. 1a. *a*, The direction of best response is colour coded and represented by arrows overlaid on the map. The length of the arrow indicates the magnitude of the direction signal; long arrows represent regions highly selective for direction of motion, short arrows represent regions that were relatively unselective. The map consists of numerous regions with high selectivity in which direction preference smoothly changes. These regions are separated by regions of low selectivity across which direction preference abruptly changes, often by 180° . The white asterisk marks the location of the recording site whose directional response is shown in Fig. 1c. The preferred direction of the site (270° , downward vertical motion) matches the directional preference of the region of the map in which it was located (downward vertical arrows). Scale bar, 1 mm. *b*, Magnitude of direction signal for the map shown in *a*. Distribution of direction preference is shown superimposed on a map where light areas represent regions with high directional selectivity and dark regions represent regions with low selectivity. The map is broken up by numerous bands, or lines of low selectivity. These bands coincide with regions of the map where direction preference also changes most rapidly (see Fig. 3).

METHODS. Maps of direction preference were produced by vectorially summing the 8 differential images (as shown in Fig. 1a) on a pixel by pixel basis according to previously described methods^{4,7}. Arrow lengths are linearly scaled to the magnitude of the resulting vector.

FIG. 3 Comparison of direction and orientation preference maps. Direction maps are shown in *a*–*c* whereas orientation maps obtained from the same cortical region are shown in *d*–*f*. Angle maps are shown in the first column (*a*, *d*); direction and orientation preference are colour coded according to the key above each map. Magnitude maps are shown in the middle column (*b*, *e*); black indicates low selectivity. Rate of change maps are shown in the last column; white indicates high rate of change. Although both orientation and direction are continuous locally and discontinuous globally, they differ significantly in the spatial organization of the discontinuities. The direction map is broken up into patches and strips by numerous lines of low selectivity (*b*) and high rate of change (*c*). In contrast, the orientation map is dominated by singularities: more punctate regions of low selectivity (*e*) and high rate of change (*f*) that correspond to the centres of pinwheels. Scale bar (in *a*), 1 mm; applies to *a*–*f*.

METHODS. Angle, magnitude, and rate of change maps were calculated according to previously described methods^{4,7}. In the magnitude maps, a cortical site with a small response magnitude may reflect one of two



conditions: 1) all or many orientations or directions evoke a strong response, or 2) one or very few nearby orientations or directions evoke a weak response. Although these two conditions cannot be distinguished, they both represent a cortical site with low selectivity to the particular stimuli within the resolution limits of our methods.

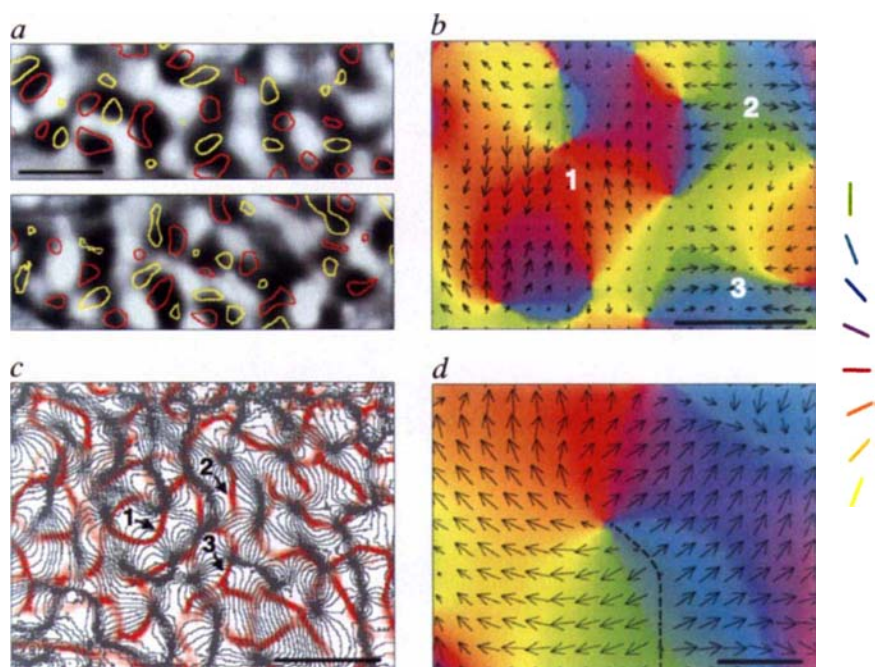
FIG. 4 Relationship between direction and orientation preference maps. *a*, Iso-direction domains for horizontal motion, 0° (red) and 180° (yellow), are shown superimposed on iso-orientation domains obtained using the same stimulus angle (vertical bars) for two animals. Iso-orientation domains activated by these stimuli appear dark in the image. Domains that prefer opposite directions of motion lie adjacent to each other and conform to the shape of the iso-orientation domains. Scale bar, 1 mm. *b*, Iso-orientation regions are subdivided into patches with opposite direction preference. Direction preference map (arrows) is superimposed on an orientation preference map (colours). Orientation preference is colour coded according to the legend shown to the right of the image. 1 is located at the centre of a region of the orientation map preferring horizontal bars (red). To the left and right of 1 are two direction patches with opposite preferred directions of motion (up/down). 2 and 3 mark orientation regions preferring vertical bars (green/blue) which are subdivided into patches preferring left/right directions of motion. These directions are orthogonal to the corresponding orientation preference. The preferred direction of motion was orthogonal, or within 45° of orthogonal, to the preferred orientation in $73.2 \pm 2\%$ of pixels ($n = 5$ animals). Sites with very weak directional selectivity (magnitude of directional response below 20% of maximum) were excluded from this calculation. This relationship is to be expected as stimulus motion was always along an axis orthogonal to its orientation. Scale bar, $500 \mu\text{m}$. *c*, Relationship between fractures in the direction preference map (red) and iso-orientation contours (grey). This image is a lower magnification view of the cortical region shown in *b* including surrounding areas. The numbers point to the corresponding sites labelled in *b*. Direction fractures bisect regions where orientation preference shifts in a gradual fashion. Although at the numbered sites, as well as other locations in the map, direction fractures intersect orientation contours at right angles, a statistically significant bias towards orthogonality was not found. The fractures also frequently intersect orientation pinwheel centres, which are seen as dark zones from which contour lines radiate. In some cases, multiple fractures radiate from a single pinwheel centre. Scale bar, 1 mm. *d*, Organization of direction preference around an orientation pinwheel. Direction preference arrows are superimposed on a colour-coded map of orientation (colour case as *b*). Direction preference shifts continuously with orientation around the pinwheel except at the fracture (dashed line) where contiguous cortical regions have opposite direction preferences. Scale bar, $200 \mu\text{m}$.

arrangement of direction selective responses in area 17 and 18 of the cat visual cortex⁸⁻¹⁰ and with the descriptions of orientation and direction preference maps in cortical area MT of the owl monkey¹⁴. Thus, orderly maps of direction and orientation preference seem to be a general feature of primary, as well as more specialized, visual cortical areas. □

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Long-range synchronization of oscillatory light responses in the cat retina and lateral geniculate nucleus

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VISUAL responses in the retina and the lateral geniculate nucleus (LGN) exhibit oscillatory patterning within a broad range of frequencies¹⁻⁹. Oscillatory activity is often associated with the synchronization of spatially distributed responses¹⁰. Here we demonstrate, with simultaneous multi-electrode recordings from the retina and the LGN, that stationary and moving light stimuli evoke in retinal ganglion cells oscillatory responses in the frequency range of 61 to 114 Hz that become synchronized over distances larger than 20 degrees of visual angle across the nasal and temporal halves of the retina. This temporal patterning of retinal responses is transmitted reliably by LGN neurons, such that stimuli crossing the vertical meridian evoke synchronous responses in the LGNs of both hemispheres. The oscillatory responses are not phase-locked to the stimulus onset, indicating that synchronization results from horizontal interactions in the retina. The occurrence of synchronization depends on global stimulus properties such as size and continuity, suggesting that temporal correlation among responses of spatially segregated ganglion cells can be exploited to convey information relevant for perceptual grouping.

Simultaneous recordings from a total of 153 pairs of recording sites were obtained with up to four independently driven micro-electrodes from neurons located either within the same LGN or in the LGNs of the two hemispheres in 17 anaesthetized cats. The receptive fields of the neurons were typically non-overlapping and, depending on the combination of recorded laminae, cells at

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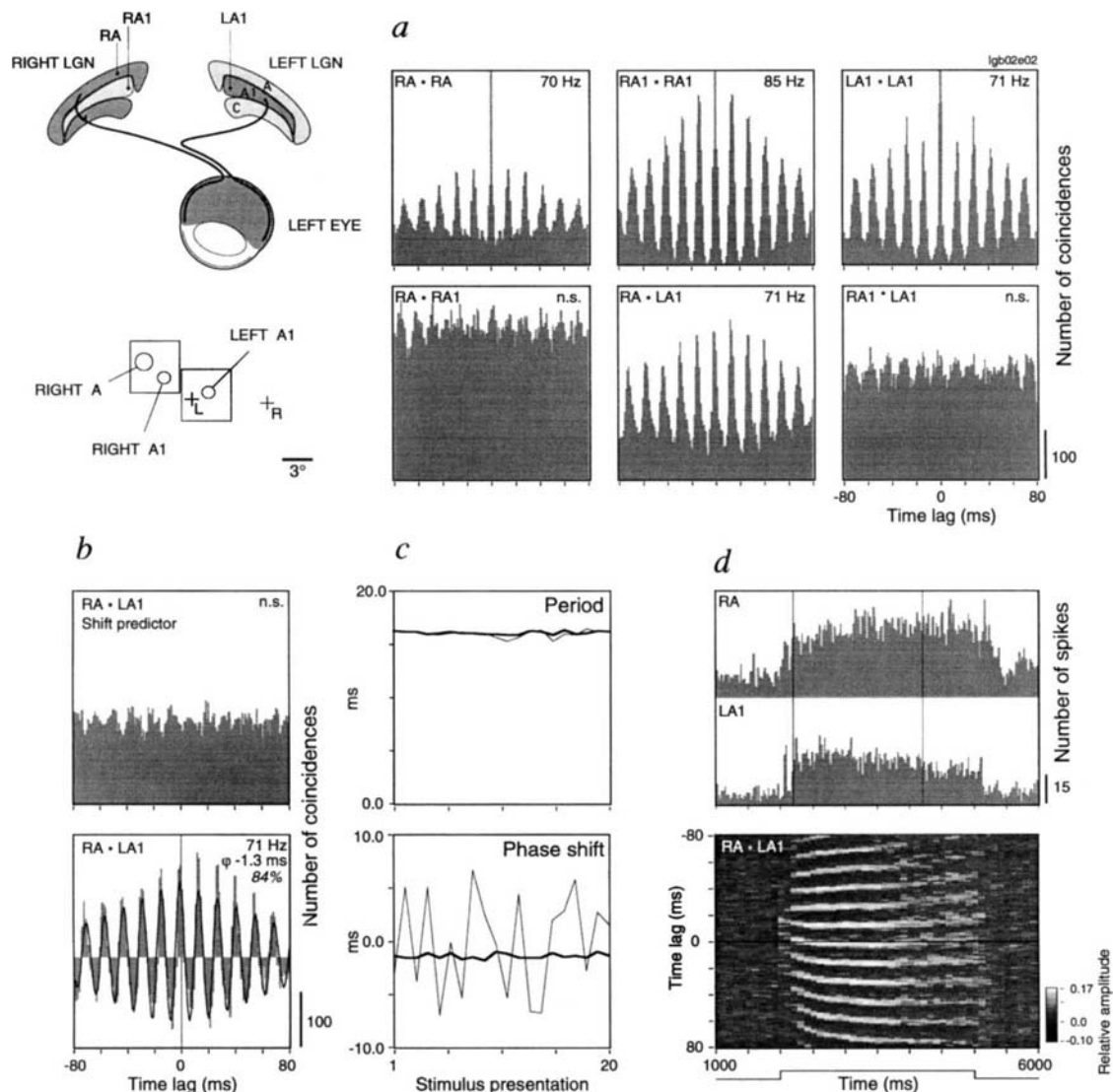


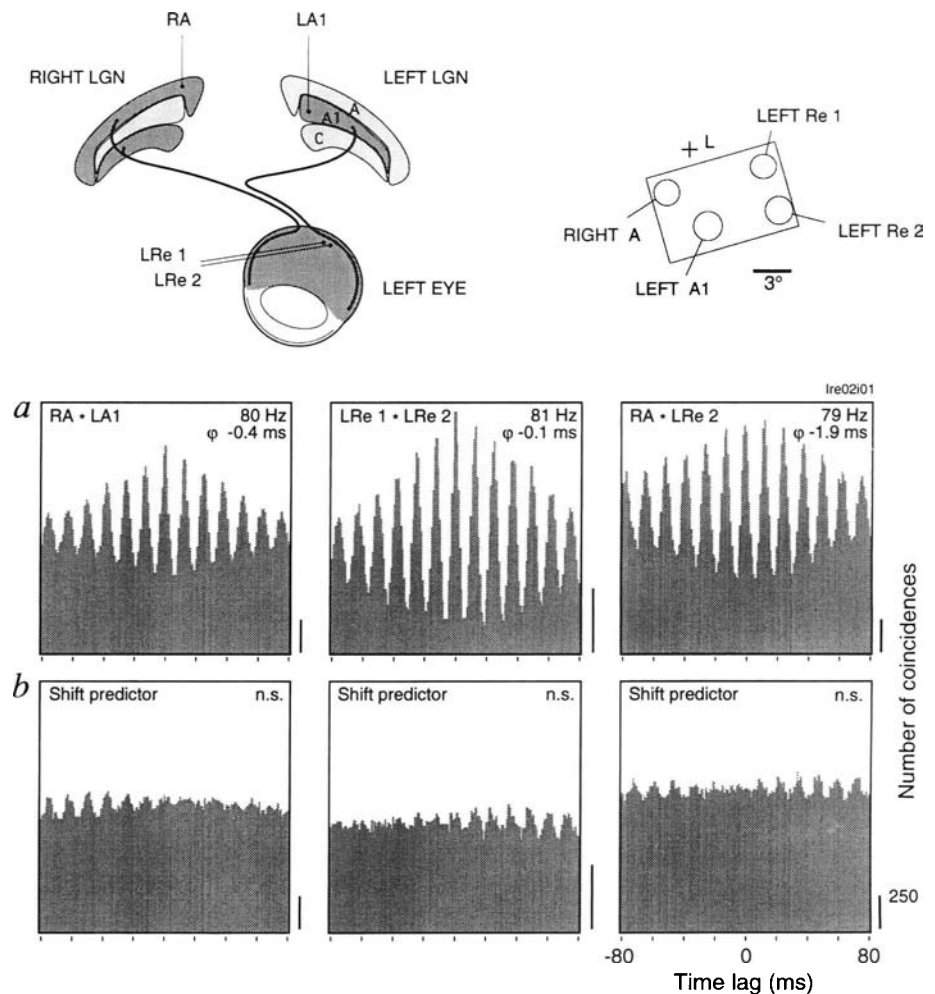
FIG. 1 Synchronized oscillatory responses of LGN cells located in different hemispheres and receiving inputs from the same eye. Multi-unit activity was recorded from two electrodes in lamina A (RA, top inset) and lamina A1 (RA1) of the right LGN, respectively. In addition, a single ON-centre cell was recorded from a third electrode in lamina A1 of the left LGN (LA1). The stimulus consisted of two stationary flashed squares (bottom inset: crosses, area centralis projections of the right (R) and left (L) eyes; squares, the light stimuli; circles, receptive fields). Notice that, after alignment of the eyes, the distance between the receptive fields RA and RA1 was about the same as between RA and LA1 (~ 6 degrees). *a*, Cumulative auto- and cross-correlograms (top and bottom, respectively) for responses to 20 stimulus presentations. As indicated by the deep periodic modulation of the auto-correlograms, the responses were oscillatory, but only those of sites RA and LA1, receiving input from the same eye, were synchronized. Oscillation frequencies were about 70 Hz for RA and LA1 but 85 Hz for RA1. *b*, The shift predictor calculated for the pair RA*LA1 was nearly flat, showing that the synchronous oscillatory responses were not phase-locked to the stimulus onset. This is confirmed by the persistence of modulation in the cross-correlogram following subtraction of the shift predictor. The damped cosine function fitted to the subtracted cross-correlogram explains 84% of its variance and indicates a phase shift (ϕ) of 1.3 ms, corresponding to a phase angle of 17 degrees. *c*, Oscillation period and phase shift of the cross-correlograms (thick lines) and shift predictors (fine lines) computed for each of the 20 successive stimulus presentations (abscissa). Note that the phase is constant for the cross correlograms but varies at random for the shift predictors. *d*, Upper panel, response histograms plotted for recording sites RA and LA1 with 25 ms bin width. The vertical lines indicate the 2 s analysis window used for the calculation of the correlograms in *a*, *b* and *c*. Lower panel, series of successive cross-correlation functions obtained through a moving window analysis (step 100 ms, analysis window 200 ms; amplitude normalized by the number of spikes in each window). Comparison of the moving window correlogram with the time course of the

responses shows that synchronization is stable throughout most of the response epoch. Notice the progressive change in the frequency of correlated oscillations during the response. Stimulation started at 2,000 ms and lasted 3 s, as depicted on the abscissa.

METHODS. Simultaneous recordings from LGN neurons were performed in lightly anaesthetized (N_2O and O_2 , 70:30%, supplemented with 0.5% halothane) and paralysed (pancuronium, $0.1 \text{ mg kg}^{-1} \text{ h}^{-1}$) adult cats, as described previously²⁴. Single- and multi-unit activity was recorded with independently controlled tungsten electrodes inserted through guide tubes into the LGN of the same or different hemispheres. For visual stimulation, stationary or moving spots, bars and gratings were generated with an optical bench (contrast, 0.75), and front-projected onto a tangent screen. In some cases, drifting gratings were generated by a computer screen with a refresh rate of 100 Hz (contrast, 0.50). Background luminance was kept around 0.4 cd m^{-2} . Auto- and cross-correlograms¹¹ accumulated over 20 stimulus presentations were computed with a resolution of 1.0 ms within 1–2-s windows, beginning 100–300 ms after stimulus onset to exclude the initial phasic component. When a computer screen was used, cross-correlograms between a photodiode signal and the neuronal responses showed that the responses were not locked to the flicker of the screen. For quantitative analysis, a damped-cosine function was fitted to the correlograms²⁵. A modulation amplitude index (the ratio between the peak amplitude of the fitted function and its offset) was taken as a measure of correlation strength. Responses were flagged as synchronous only if the following criteria were fulfilled: modulation in the correlogram persisted after subtraction of the shift predictor; the fitted function explained at least 20% of the correlogram variance; its central and first satellite peaks had Z-scores larger than 3; and the modulation amplitude index was equal to or greater than 0.2. This latter criterion was necessary to exclude correlograms in which modulations resulting from chance correlation of oscillatory signals with similar frequency had not been averaged out completely over 20 stimulus presentations.

FIG. 2 Synchronized oscillatory responses in the retina account for synchronization of responses in the LGN across the hemispheres. Multi-unit activity was recorded simultaneously from two separate sites in the retina of the left eye (LRe1 and LRe2), and in lamina A of the right (RA) and lamina A1 of the left LGN (LA1), both laminae receiving input from the left eye. A large flashed stimulus evoked strong oscillatory responses at all four recording sites that were synchronized with one another. *a*, Cumulative cross-correlograms between responses recorded from the two LGNs (left panel), the two sites in the temporal retina of the left eye (centre panel), and lamina A in the right LGN and one of the recording sites in the temporal retina (right panel). Note the similar oscillation frequencies of about 80 Hz at all sites. *b*, Shift predictors corresponding to each pair of recording sites were flat.

METHODS. For simultaneous LGN and retinal recordings, the left eye was immobilized by suturing it to a metallic ring attached to the stereotaxic frame, under deep anaesthesia²⁶. Single- and multi-unit activity was recorded from the retina with two tungsten electrodes inserted into the eye through a guide tube. The electrodes could be moved independently and placed under visual control at the desired location. Following the positioning of the electrodes in the retina, the LGNs in the two hemispheres were recorded as described above.



different recording sites were driven by the same eye or by different eyes. Interactions between the neurons were studied by computing auto- and cross-correlograms¹¹ for responses to stationary flashed or moving bars or gratings. To distinguish correlations due to neuronal interactions from stimulus-induced covariations of firing rates, we always computed control correlograms between responses evoked by successive presentations of the stimulus (shift predictors¹²). Auto-correlation analysis revealed that a substantial fraction of responses (48%, $N = 1,271$ correlograms) exhibited oscillatory patterning, ranging in frequency from 38 to 125 Hz. Oscillatory responses could be synchronized with a precision in the millisecond range irrespective of whether the cells were distributed within or across laminae of one LGN or across the LGNs of the two hemispheres. An example of interhemispheric correlation between oscillatory responses is shown in Fig. 1 for two recording sites located in lamina A of the right and lamina A1 of the left LGN, which receive input from the left eye. The receptive fields were separated by ~ 6 degrees across the vertical meridian. The responses were strongly oscillatory and precisely synchronized, without phase-locking to the onset of stimulus (Fig. 1*b, c*).

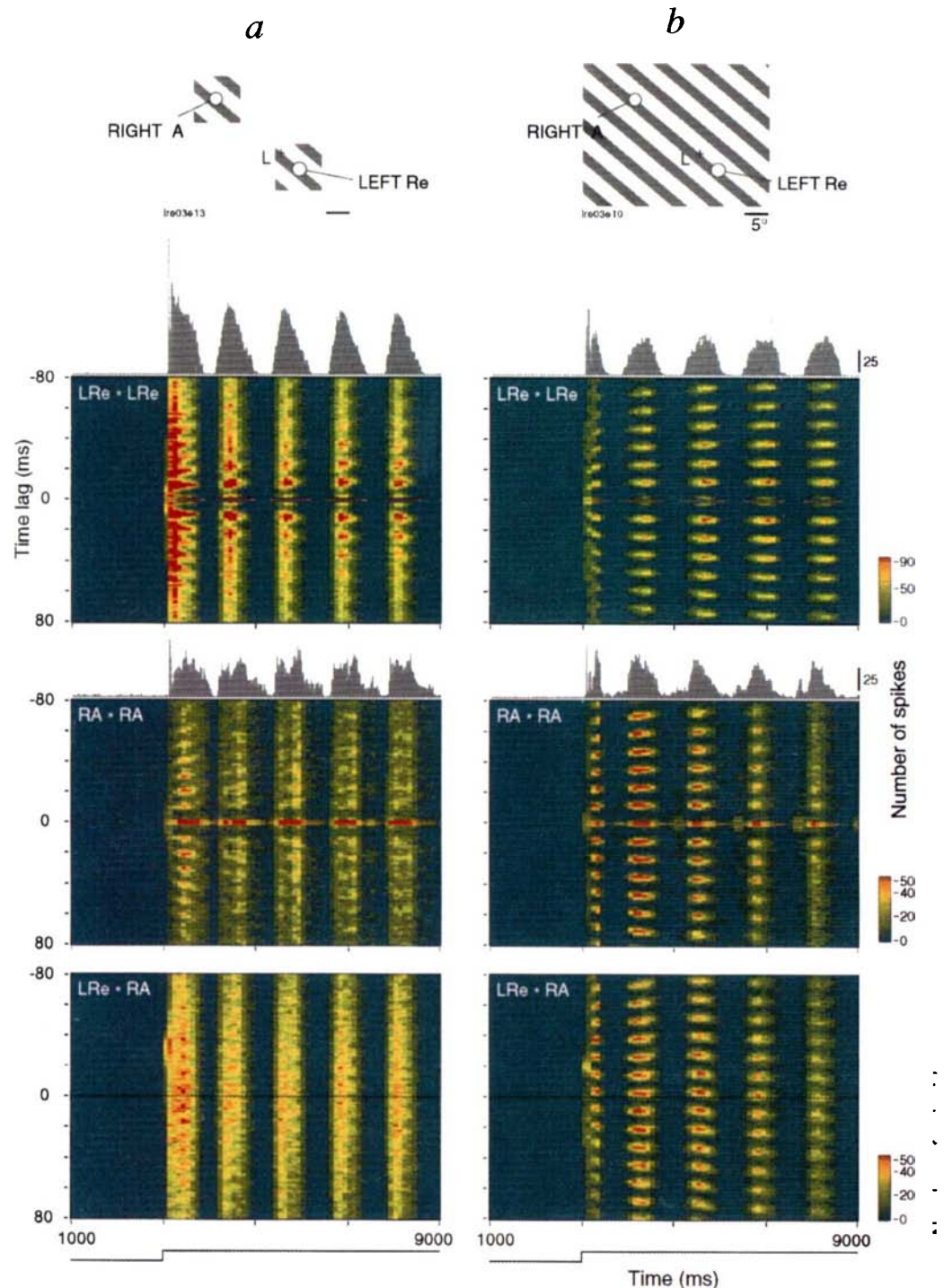
All cross-correlograms with a significant centre peak reflecting response synchronization exhibited strong periodic modulation, suggesting that correlation was mediated by oscillatory activity, with a mean frequency of 81 ± 17 Hz ($N = 36$ pairs of recording sites). The phase angle of synchronization was smaller than 45 degrees in about half the cases (mean 49 ± 40 degrees, range from 2 to 137 degrees; mean phase shift 1.8 ± 1.6 ms). The correlations were strong (mean modulation amplitude 0.9 ± 0.7), and varied little through most of the response epoch (Fig. 1*d*). Both the oscillation frequency of the responses and the phase shift of the

synchronization remained remarkably constant within and across trials (Fig. 1*d, c*).

Oscillatory discharges and their synchronization depended on visual stimulation. Small stationary or moving stimuli restricted to receptive field centres were poor at evoking oscillatory patterns, although they commonly elicited stronger responses. Oscillations were induced most readily by large stationary stimuli or moving gratings, and occurred even after global changes in luminance, suggesting the involvement of centre-surround interactions in the generation of rhythmic activity. Although synchronization was always associated with oscillatory activity, the reverse was not the case. Pairs of recording sites receiving input from different eyes showed no correlation between their oscillatory responses (Fig. 1*a*). Indeed, synchronization was essentially restricted to cells driven by the same eye. Of 84 pairs of recording sites receiving inputs from the same eye, 33 showed synchronous responses, and 10 of these were distributed across the hemispheres. In contrast, none of the 35 pairs distributed across the hemispheres and receiving inputs from different eyes showed response synchronization.

Synchronous oscillations in the LGN could in principle be of cortical, thalamic or retinal origin. Synchronization by feedback projections from the cortex is unlikely because oscillatory responses in the cortex generally occur at lower frequencies (30–50 Hz) and exhibit greater variability in frequency and duration. If cortical responses synchronize, correlations tend to be less precise and correlation strength fluctuates¹⁰. Accordingly, the previously described cortically mediated synchronization of LGN responses is much less precise and not based on high-frequency oscillations¹³. Thalamic mechanisms can be excluded because they cannot account for interhemispheric synchroniza-

FIG. 3 Stimulus-dependent synchronization between oscillatory responses in the retina and the LGN. Two ON-cells were recorded, one from the temporal retina of the left eye, and the other from lamina A of the right LGN, which receives input from the nasal retina of the left eye. Receptive fields were separated by 26 degrees across the vertical meridian. Responses were evoked by a moving grating that was either limited to two apertures positioned over the two receptive fields (*a*) or extended over a large portion of the visual field (*b*). Top and middle panels, auto-correlation functions obtained through a moving window analysis, aligned with the response histograms (step 100 ms, analysis window 200 ms). Oscillatory patterning of responses was more pronounced when the stimulus covered a large area, although firing rate was reduced (see response histograms above panels, 25 ms bin width). Bottom panels, cross-correlation functions. Synchronization is significant only for the large stimulus (*b*). Synchronous oscillatory activity develops soon after the transient response to the onset of the stimulus, and then occurs right from the beginning of the responses to subsequent cycles of the grating. Notice the progressive change in the frequency of correlated oscillations throughout stimulation. This change follows the same time course as the change in oscillation frequency during the response to stationary stimuli documented in Fig. 1*d*. Spatial frequency of the grating, 0.2 cycles per deg; contrast, 0.50; mean screen luminance before stimulus, 0.7 cd m^{-2} ; during stimulus, 1.3 cd m^{-2} .



tion. But the restriction to responses driven by the same eye strongly suggests a retinal mechanism for synchronization. To examine this possibility, we made simultaneous multi-electrode recordings from the LGN and from retinal ganglion cells in six additional cats. As in the LGN, most of the ganglion cell responses were oscillatory, with frequencies ranging from 61 to 114 Hz (mean $88 \pm 10 \text{ Hz}$, $N = 458$ correlograms). Synchronous oscillations were observed in 20 out of 32 pairs of recording sites in the retina (62%; mean frequency $82 \pm 13 \text{ Hz}$; mean phase shift 1.0 ± 1.1 , ranging from 0 to 2.8 ms). Of 63 pairs of recording sites between one retina and LGN laminae receiving input from this eye, 26 pairs (41%) showed synchronized oscillatory responses. Figure 2 illustrates an example of simultaneous multi-unit recordings from two sites in the retina of the left eye

with non-overlapping receptive fields and two further sites in the LGNs of the two hemispheres receiving input from the left eye. A large flashed stimulus evoked precise, non-stimulus-locked oscillatory responses that were synchronous across all four sites. As there are no feedback connections from the LGN to the retina, this proves that both the oscillatory patterning of the responses as well as their synchronization occur in the retina. Moreover, it indicates that the LGN cells reliably transmit the temporal patterning of their retinal input.

Synchronous oscillatory responses were observed for recording sites of same-eye input in the LGN for receptive field separations up to 15 degrees, the largest distance examined in the LGN, without indication of a distance-dependent decrement of the modulation amplitude. Similarly strong synchronization was

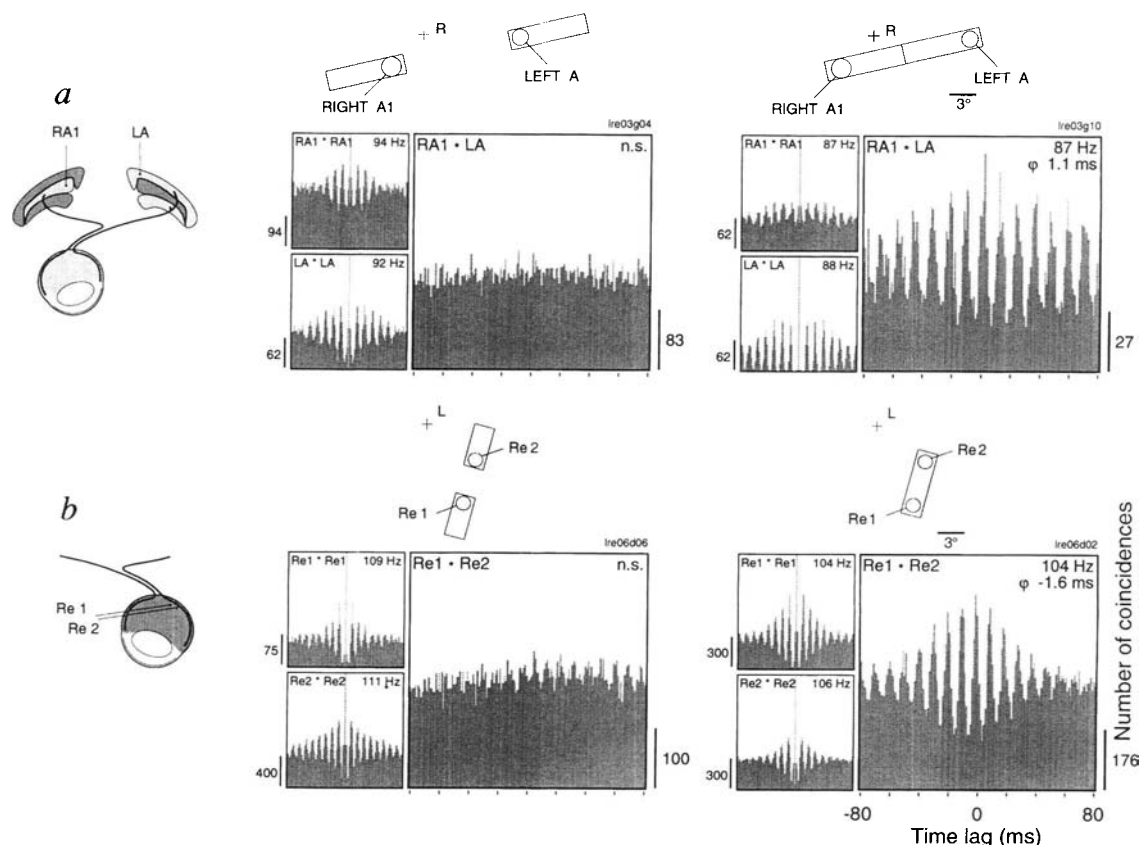


FIG. 4 Dependence of synchronization in the LGN and in the retina on stimulus configuration. *a*, Multi-unit activity was recorded in lamina A1 of the right, and a single ON-cell in lamina A of the left LGN, with receptive fields separated by 15 degrees across the vertical meridian, both driven from the right eye. *b*, Two single ON-cells with receptive fields separated by 6 degrees were recorded in the left retina. The cells were activated by rectangular light stimuli that were either widely separated or extended across the area between the receptive fields. When the area between the

receptive fields was not stimulated, the oscillatory responses were not correlated even though responses at both sites had similar oscillation frequencies (left panels). When the stimuli were continuous, covering the area between the receptive fields, the oscillatory responses in the LGN (*a*) and in the retina (*b*) became highly correlated (right panels). Auto- (smaller boxes) and cross-correlations are plotted with the same time shift of 80 ms.

observed between retinal and LGN responses for sites whose receptive fields were separated by as much as 26 degrees. In the case illustrated in Fig. 3, strong synchronous oscillatory responses were induced with moving gratings covering ~ 40 degrees of the visual field. Synchronous oscillations developed immediately after the transient response to stimulus onset, and for the subsequent responses, occurred right from the beginning of each discharge cycle, with a gradual decrease in oscillation frequency. When the same stimulus was subdivided to cover only the regions of the two receptive fields, the oscillatory patterning was reduced even though the firing rates were comparable, and there was no synchronization. This suggests that the stimulus organizes a large population of cells in the retina into ensembles that cooperate across long distances.

For this long-range synchronization to occur the responses must be evoked with continuous stimuli (Fig. 4*a*). Two spatially contiguous stimuli covering the receptive fields of two LGN cells induced strong synchronization of the oscillatory responses. However, when the same stimuli were repositioned to cover the receptive fields but not the intermediate area, synchronization was disrupted without a decrease in oscillation strength of the individual responses, indicating that continuity, and not stimulus size, was responsible for synchronization. The same relation between stimulus continuity and response synchronization holds for the retina (Fig. 4*b*), confirming that the stimulus-dependency of synchronization in the LGN has a retinal origin.

Oscillatory activity covering a broad range of frequencies has been described previously both in the retina^{1,3-5} and the LGN^{1,4,6-9}. Most studies have dealt with spontaneous activity, and reports on

light-evoked oscillations have been controversial^{1,3,4,6}. Our results extend these observations by providing direct evidence that spatially structured light stimuli evoke high-frequency oscillations in retinal ganglion cells that are transmitted reliably by LGN neurons. Reports on correlated firing in the LGN^{4,13,14} and the retina¹⁵⁻¹⁸ have been limited so far to closely spaced neurons. In the retina, correlation strength depended on the amount of overlap of the RFs of the cells, from which it was concluded that correlation results from shared input¹⁸. Our results imply a different mechanism by demonstrating synchronization over large distances, which relies on oscillatory patterning of the responses and depends critically on the spatial configuration of the stimuli. Synchronization might arise from phase-locking of spatially adjacent oscillatory modules extending horizontally across the retina. The required lateral interactions could be mediated by the distributed, partially reciprocal connections among amacrine cells and ganglion cells, or by gap-junctions linking amacrine cells and a subpopulation of ganglion cells¹⁹⁻²².

Because long-range synchronization in the retina depends on stimulus properties such as size and continuity, it might signal basic spatial relationships among stimulus features, which could be exploited in perceptual grouping at higher levels of visual processing. Synchronous input from the LGN to both cortical hemispheres could facilitate interhemispheric synchronization of cortical responses mediated by the corpus callosum²³ and thereby contribute to the binding of features of objects extending across the vertical meridian. It needs to be emphasized, however, that these subcortical synchronization mechanisms alone cannot account for the previously described synchronization among

cortical neurons¹⁰ although they are likely to contribute. Also, the present data do not exclude additional modulation of synchrony among LGN cells by corticofugal effects. Simultaneous recordings from LGN cells and cortical neurons revealed that cortical synchronization can be both related and unrelated to thalamic synchronization depending on stimulus configuration (unpublished observations). Thus, synchronization of neuronal responses appears to occur at several different levels of visual processing including the retina, and where feedback loops exist the various mechanisms can interact with one another. □

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Inhibition of monoamine oxidase B in the brains of smokers

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THE massive health problem associated with cigarette smoking is exacerbated by the addictive properties of tobacco smoke and the limited success of current approaches to cessation of smoking¹. Yet little is known about the neuropharmacological actions of cigarette smoke that contribute to smoking behaviour, or why smoking is so prevalent in psychiatric disorders² and is associated with a decreased risk of Parkinson's disease³. Here we report that brains of living smokers show a 40% decrease in the level of monoamine oxidase B (MAO B; EC 1.4.3.4) relative to non-smokers or former smokers. MAO B is involved in the breakdown of dopamine, a neurotransmitter implicated in reinforcing and motivating behaviours as well as movement. MAO B

inhibition is therefore associated with enhanced activity of dopamine⁴, as well as with decreased production of hydrogen peroxide, a source of reactive oxygen species⁵. We propose that reduction of MAO B activity may synergize with nicotine to produce the diverse behavioural and epidemiological effects of smoking.

We compared brain MAO B in smokers and non-smokers using positron emission tomography (PET) and deuterium substituted [¹¹C]L-deprenyl ([¹¹C]L-deprenyl-D₂), a radiotracer which binds irreversibly to MAO B, labelling it *in vivo*^{6,7}. The influx constant (K_i) (ref. 8), which is a function of MAO B concentration, was used to quantify MAO B concentrations in different brain regions⁷. K_i was reduced in all brain regions examined (Table 1 and Fig. 1a for graphical analysis for the thalamus). For example, the average influx constant for the basal ganglia is 0.15 ± 0.05 and 0.25 ± 0.02 cubic cm brain per ml plasma per min for the smokers and non-smokers, respectively ($P < 0.0001$, unpaired *t*-test).

We also measured the blood-to-brain transfer constant (K_1) for [¹¹C]L-deprenyl-D₂. The constant did not differ between smokers and non-smokers, indicating that smoking does not change [¹¹C]L-deprenyl-D₂ delivery to the brain (see Table 1 for K_1 of the whole brain) confirming that the changes in radiotracer trapping are not due to changes in radiotracer delivery or brain blood flow. This result is consistent with the report that brain blood flow does not differ between young smokers (mean age 55 years) and non-smokers⁹.

To assess the specificity of the effect, brain glucose metabolism was also measured using 2-deoxy-2-[¹⁸F]fluoro-D-glucose¹⁰. Metabolism in the specific regions examined, and in the whole brain, did not differ between smokers and non-smokers. The average values ($\pm$ s.d.m.) for the whole brain (see Table 1) were 32.9 ± 2.1 and 33.3 ± 3.2 μ mol glucose per 100 g per min for the non-smokers ($n = 6$) and the smokers ($n = 7$), respectively. There was no association between brain glucose metabolism and MAO B concentration. Both this result and the similarity in K_i between groups indicate that inhibition of MAO B is a selective effect of smoking and is not caused by variability in blood flow or resting metabolism. The assessment of selectivity with respect to other neurochemical processes requires further investigation. Acute nicotine administration has been reported to reduce brain glucose metabolism in humans¹¹.

To determine the degree to which cigarette smoking inhibits MAO B relative to the degree of inhibition produced by therapeutic MAO B inhibition, we treated one of the subjects with a therapeutic dose of the selective MAO B inhibitor, L-deprenyl (10 mg day⁻¹) for 1 week. This dose is known to inhibit brain MAO B by >90% (ref. 12). L-Deprenyl treatment reduced the value of the influx constant by 86% relative to the baseline value (average reduction for the five brain regions) (Table 1 and Fig. 1b for graphical analysis for the thalamus). In contrast, the value of K_i was similar before and after L-deprenyl treatment (0.410 and 0.450 for baseline and L-deprenyl treatment, respectively). One of the smokers (subject 11) had brain MAO B levels comparable with the subject (17A) who was treated with L-deprenyl. Comparative influx constant images for a non-smoker, a smoker and an individual receiving L-deprenyl are shown in Fig. 2.

MAO B levels were comparable for former smokers and non-smokers (Table 1 and Fig. 3). This indicates that the differences in MAO B between smokers and non-smokers are due to pharmacological and not genetic factors. Data from the literature also support a pharmacological effect. For example, platelet MAO B, which has been reported to be reduced in cigarette smokers¹³, returns to normal values after smoking cessation¹⁴. In addition, cigarette smoke and extracts of cigarette smoke inhibit MAO both *in vivo* in rodent brain^{15,16} and *in vitro*^{17,18}, and saliva from cigarette smokers inhibits MAO¹⁷.

In our study, the time interval between last cigarette and the PET scan ranged from 1.7–12 hours. As the time course of MAO B recovery after withdrawal from cigarettes is not known, it is possible that we have underestimated the degree of chronic inhibition in the cigarette smoker where the recycle time may be 1 h or less. It has been reported that cigarette smoke irreversibly

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inhibits MAO *in vitro*¹⁷. If inhibition is irreversible *in vivo*, then the effects of cigarette smoking would be predicted to be cumulative because the synthesis rate of human brain MAO B is slow with a half-life of about 40 days¹⁹.

MAO B levels in the smokers varied over a wider range than in the non-smokers. For example, the average coefficients of variation for the five brain regions and the whole brain were 35% and

12.5% for smokers and non-smokers, respectively. Factors such as cigarette type, daily smoking dose, number of years of smoking, time interval between last cigarette and PET scan, and smoking behaviour may all contribute to the degree of MAO B inhibition and need to be investigated.

The mechanism(s) of MAO inhibition by cigarette smoke are not known. It has been proposed that cyanomethylation (brought

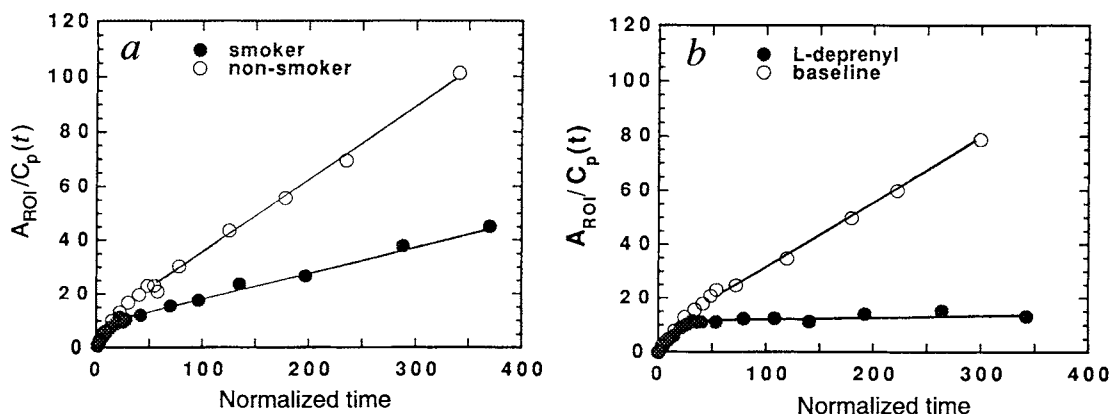
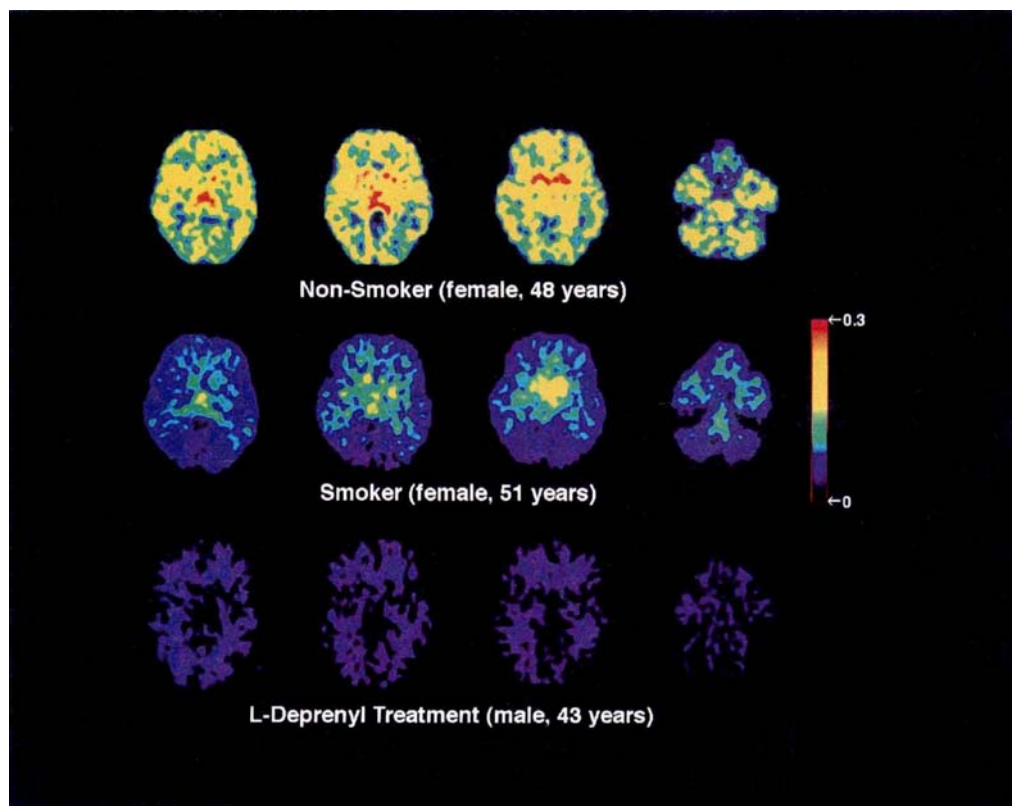


FIG. 1 *a*, Graphical analysis⁸ of results from the thalamus for a non-smoker (subject 6: female, 48 years) and a smoker (subject 13: female, 51 years). $A_{ROI}/C_p(t)$ refers to the ratio of ^{11}C concentration in a region of interest (ROI) from the thalamus (A_{ROI}) to that of the plasma concentration of tracer (C_p) at time (t). Normalized time refers to the ratio of the arterial plasma concentration–time integral to C_p . The influx constant (K_1) is the slope of the plot. Influx constants are 0.29 and 0.11 cubic cm brain per ml plasma per min for the non-smoker and the smoker, respectively. Whole-brain values of K_1 are

0.48 for each subject. *b*, The effect of treatment with a therapeutic dose of the MAO B inhibitor drug L-deprenyl (10 mg d^{-1}) on the influx constant in thalamus of one of the subjects (subject 17 and 17A, a former smoker (male, 43 years) before and after L-deprenyl treatment). The influx constants are 0.24 and 0.017 cubic cm brain per ml plasma per min at baseline and after L-deprenyl, respectively. Whole-brain values of K_1 are 0.41 and 0.45 at baseline and after L-deprenyl, respectively.

FIG. 2 Influx constant images for a non-smoker (Subject 6: top row), a smoker (Subject 13; middle row) and a subject who had received L-deprenyl (Subject 17A: 10 mg d^{-1}) for 1 week (bottom row). The same 4 planes left to right are 4.7, 3.5, 2.8 and 1.4 cm superior to the canthomeatal line and show the thalamus, basal ganglia, ventral striatum and cerebellum for each subject. The colour scale is shown with red corresponding to $K_1 = 0.3$ cubic cm brain per ml plasma per min. The K_1 values for the thalamus are 0.29 for the non-smoker, 0.11 for the smoker and 0.017 for the subject who received L-deprenyl.

METHODS. Influx constant images were generated using graphical analysis⁸ applied to the time course of radioactivity at each pixel in the reconstructed image falling within the brain. The part of the image falling within the brain was determined by masking the reconstructed image with a binary representation of the brain shape for each plane. These binary images were obtained by averaging the first five scans (up to 6 min) and applying a cut-off of $0.25 \times$ the maximum value in the plane to separate brain from non-



brain. All image calculations were performed using pixels falling within this binary image. The brain area was filled in using morphological transformations³⁰.

about by formaldehyde and cyanide in smoke) of the reactive amino groups in the MAO protein may reduce its catalytic activity²⁰. Nicotine does not inhibit MAO B in physiologically relevant concentrations^{13,18,21}, and hydrazine and phenylpyridine, two other components of cigarette smoke, also do not inhibit the enzyme *in vivo*^{18,21}.

Although the functional and neurochemical consequences of the presence of MAO B and of long-term partial inhibition of human brain MAO B are not well understood⁴, L-deprenyl therapy has been reported to reduce the rate of progression of Parkinson's disease²² and to improve cognition in humans²³, and to increase life span and improve learning performance in rats²⁴. This has been attributed to enhanced dopaminergic neurotransmission as biochemical studies have found that chronic L-deprenyl treatment results in an elevation of basal dopamine release in the corpus striatum²⁵. MAO B inhibition may also have secondary effects on other neurotransmitters such as serotonin and noradrenaline as a result of dopamine's complex interactions²⁶.

As nicotine stimulates dopamine release and an elevation in dopamine in the nucleus accumbens is associated with reinforcement²⁷, our study raises the question as to whether MAO B inhibition by cigarette smoke synergistically enhances nicotine-induced dopaminergic neurotransmission. Although electrophysiological measurements in animals show that L-deprenyl potentiates striatal dopaminergic neurotransmission induced by dopamine agonists²⁸, it remains to be determined whether MAO B inhibition is involved in altering the reinforcing properties of drugs of abuse, including nicotine, in the human brain.

Brain MAO B inhibition by cigarette smoke may contribute to the lower incidence of Parkinson's disease among cigarette

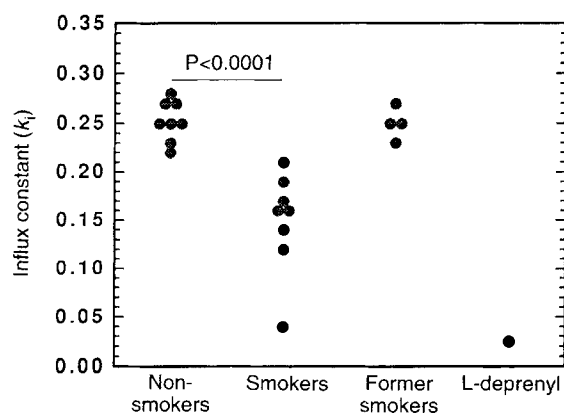


FIG. 3 Effect of smoking status on the influx constant for the basal ganglia for non-smokers ($n = 8$), smokers ($n = 8$) and former smokers ($n = 4$). The MAO B value for a subject who had received a therapeutic dose of L-deprenyl (10 mg d^{-1}) for 1 week is provided for comparison. Smokers had significantly lower MAO B than non-smokers ($P < 0.0001$, unpaired *t*-test, two tailed). Former smokers had values comparable to the non-smokers.

smokers by reducing the levels of hydrogen peroxide, a by-product of MAO oxidation and a source of reactive oxygen species⁵. This may be one of the mechanisms for the delay of the progression of Parkinson's disease with L-deprenyl treatment²². MAO B inhibition by cigarette smoke could also afford protection from

TABLE 1 Smoking history of subjects and model parameters relating to MAO B concentration

Influx constant (K_1 : cubic cm brain per ml plasma per min)												
Subject	Gender	Age	Packets per day (y)	Time interval	GM (GL)	K_1 (GL)	GL	BG	TH	CB	CING	FR
Non-smokers												
1*	male	27	NA		34.8	0.42	0.10	0.22	0.19	0.10	0.16	0.13
2*	male	34	NA		35.4	0.56	0.11	0.25	0.21	0.15	0.15	0.14
3	female	34	NA			0.60	0.11	0.27	0.24	0.12	0.15	0.14
4*	male	42	NA		33.1	0.44	0.11	0.25	0.24	0.14	0.18	0.15
5	female	49	NA			0.59	0.14	0.27	0.27	0.11	0.17	0.15
6*	female	48	NA		30.4	0.48	0.15	0.28	0.29	0.17	0.14	0.17
7*	male	64	NA		30.71	0.31	0.10	0.23	0.23	0.13	0.16	0.14
8*	male	67	NA		32.9	0.40	0.12	0.25	0.24	0.11	0.16	0.13
					32.9 ± 2.1	0.49 ± 0.11	0.12 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.13 ± 0.02	0.16 ± 0.01	0.15 ± 0.01
Smokers												
9*	male	23	0.5(3)	12 h	35.5	0.44	0.09	0.10	0.16	0.10	0.10	0.10
10*	male	31	1(10)	4 h	34.4	0.40	0.08	0.16	0.18	0.08	0.14	0.10
11*	male	31	1.5-2(18)	1.7 h	32.3	0.42	0.04	0.04	0.04	0.03	0.05	0.03
12*	male	44	1(30)	4 h	38.6	0.30	0.06	0.12	0.11	0.07	0.07	0.06
13*	female	51	1(30)	2.5 h	32.8	0.48	0.06	0.14	0.11	0.06	0.10	0.07
14*	female	51	1.5(21)	2.3 h	30.5	0.49	0.09	0.17	0.18	0.08	0.13	0.11
15	male	56	1(38)	2 h		0.62	0.07	0.16	0.15	0.07	0.08	0.09
16*	male	62	1.5(42)	2 h	29.1	0.39	0.10	0.21	0.21	0.13	0.16	0.14
					33.3 ± 3.2	0.44 ± 0.09	0.07 ± 0.02	0.15 ± 0.05	0.14 ± 0.05	$0.08 \pm .03$	$0.10 \pm .04$	$0.09 \pm .03$
Former smokers												
17	male	43	1(4)	21 y		0.41	0.11	0.25	0.24	0.13	0.17	0.15
18*	male	50	1.75(7)	25 y	39.8	0.48	0.14	0.25	0.26	0.14	0.17	0.15
19	male	63	1(37)	7 y		0.35	0.12	0.23	0.22	0.12	0.15	0.14
20*	male	86	1.5(60)	10 y	27.5	0.4	0.14	0.27	0.26	0.14	0.19	0.17
						0.41 ± 0.05	0.13 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	$0.13 \pm .01$	$0.17 \pm .02$	$0.15 \pm .01$
L-deprenyl treatment												
17A	male	43	1(4)	21 y		0.45	0.027	0.025	0.017	0.014	0.022	0.031

Our studies followed the guidelines of the Human Subjects Research Committee at Brookhaven National Laboratory. Subjects were injected intravenously with [^{11}C]L-deprenyl- D_2 ; 6.9–8.5 mCi (2–12 μg)⁷. Brain glucose metabolism (μmol glucose per 100 g per min) was measured in 15 subjects (indicated with *) with ^{18}F FDG as described previously¹⁰. Subjects with a past or present history of drug abuse (except cigarettes, as indicated) were excluded. Subjects were free from neurological, psychiatric and cardiovascular disease and were not taking any medication except for subject 16 who was receiving 1 aspirin per day and cardizem (480 mg d^{-1}) for treatment of hypertension. Subject 17 was scanned before and after treatment with L-deprenyl (10 mg d^{-1} , 17A) for 1 week to assess complete MAO B inhibition. Time-activity data for all brain regions were accumulated for 60 min and an arterial plasma input function for [^{11}C]L-deprenyl- D_2 was measured as described previously⁷. Regions of interest were selected as described previously⁷. For the thalamus, basal ganglia and frontal cortex, the values from right and left regions were averaged. An appropriate blood volume correction (4%) was subtracted from the PET data before parameter optimization. For each subject, the plasma to brain transfer constant (K_1) was obtained using a three-compartment model⁷. The relative MAO B concentration for different brain regions and for the whole brain was measured by calculating an influx constant (K_1) using graphical analysis for tracers which are unidirectionally transferred from plasma to tissue⁸. In this analysis it is assumed that non-specific binding occurs very rapidly, much more rapidly than transport or binding to the enzyme so that it is always in steady state. This analysis strategy has been applied previously to [^{11}C]L-deprenyl- D_2 kinetics in human brain⁷. Time-activity data from brain and from plasma have also been analysed using a three-compartment model and the model terms k_3 and k_3' differ significantly between smokers and non-smokers ($P < 0.03$ and 0.0002 for k_3 and for k_3' respectively for the whole brain). Both of these terms are also corrected with the influx constant K_1 ($r^2 = 0.77$; $P < 0.0001$ and $r^2 = 0.89$; $P < 0.0001$ for k_3 and for k_3' for the whole brain). There is no correlation between K_1 and K_1' ($r^2 = 0.074$; NS). [^{11}C]L-deprenyl- D_2 has a reduced rate of trapping in brain relative to [^{11}C]L-deprenyl and thus K_1 is relatively insensitive to changes in blood flow and is therefore a more stable measure of relative MAO B concentration than k_3 obtained from a three-compartment model. For example, a 15% decrease in K_1 in the basal ganglia gives a 4.4% and 5% decrease in K_1 in the basal ganglia and whole brain, respectively. In the graphical analysis for irreversible systems, the slopes (K_1) were taken as an average of slopes between 6 and 45 min and 6 and 55 min. The number of packets of cigarettes smoked per day is shown with the number of years of smoking in parentheses. Time interval refers to the time between the last cigarette and the PET scan. The influx constant (K_1) was significantly different in all brain regions for smokers compared with non-smokers ($P < 0.002$) but not for current smokers and former smokers and there was no significant affect of subject's age or gender on K_1 . The plasma to brain transfer constant (K_1) and brain glucose metabolism (GM) for the different brain regions did not differ between groups (data shown only for the global region; the whole brain). Abbreviations: GL (global), BG (basal ganglia), TH (thalamus), CB (cerebellum), CING (cingulate gyrus) and FR (frontal cortex).

exposure to chemical compounds such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which are oxidized by MAO B to produce neurotoxic product(s). Cigarette smoke has been reported to attenuate the neurotoxic effects of MPTP¹⁵. Inhibition of MAO B by 40% is required for reduction of MPTP toxicity²⁹ which is similar to the average MAO B reduction we observed in cigarette smokers.

Cigarette smoking poses a massive, yet potentially preventable, public health problem. But the development of more effective therapies for smoking cessation requires a better understanding of the neuropsychopharmacological basis of why people smoke. This report links the epidemiological characteristics of cigarette smoking and studies in animals, by direct examination in the relevant clinical material, the living cigarette smoker. It supports the hypothesis that MAO B inhibition may contribute to some of the epidemiological characteristics of cigarette smoking^{16,20} and suggests that MAO B inhibitor drugs may be useful as an adjunct in smoking cessation.

Note added in proof: It has recently been reported that the reversible MAO A inhibitor moclobemide facilitates the cessation of smoking in highly dependent smokers³¹. □

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Disruption of the nuclear hormone receptor ROR α in staggerer mice

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HOMOZYGOUS staggerer (sg) mice show a characteristic severe cerebellar ataxia due to a cell-autonomous defect in the development of Purkinje cells^{1,2}. These cells show immature morphology, synaptic arrangement, biochemical properties and gene expression, and are reduced in numbers^{3–12}. In addition, sg heterozygotes show accelerated dendritic atrophy and cell loss¹³, suggesting that sg has a role in mature Purkinje cells. Effects of this mutation on cerebellar development have been studied for 25 years, but its molecular basis has remained unknown. We have genetically mapped staggerer to an interval of 160 kilobases on mouse chromosome 9 which was found to contain the gene encoding ROR α , a member of the nuclear hormone-receptor superfamily. Staggerer mice were found to carry a deletion within the ROR α gene that prevents translation of the ligand-binding homology domain. We propose a model based on these results, in which ROR α interacts with the thyroid hormone signalling pathway to induce Purkinje-cell maturation.

We set out to identify the defective gene in *staggerer* by positional cloning, using genetic and physical mapping (Fig. 1). Apart from some large chromosomal deletions (such as Df 10DFoD; Fig. 1), there is only one allele of *sg*. We mapped this allele relative to simple sequence length polymorphisms (SSLPs) from the Whitehead/MIT map¹⁴ in 2,497 informative meioses. Marker loci that remained tightly linked to *sg* after 1,000 meioses were used to isolate clones from yeast artificial chromosomes (YACs)¹⁵. Genetic mapping localized *sg* to a 610-kilobase (kb) YAC and subsequently to a 162-kb bacterial artificial chromosome (BAC), 28A1.

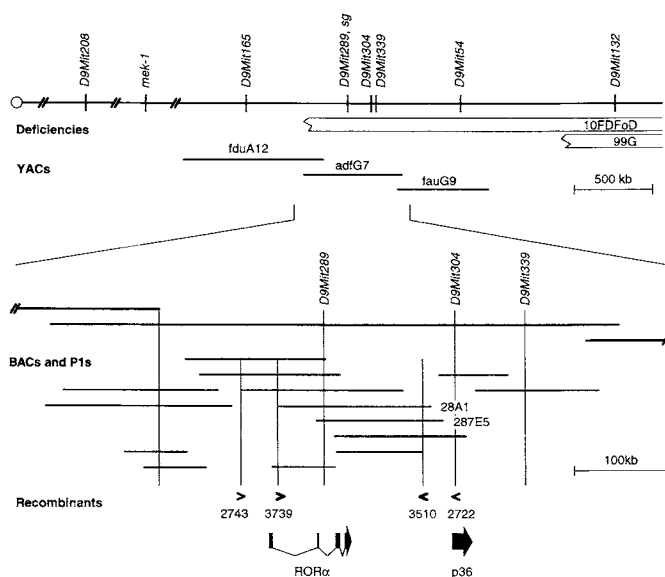
In the course of sequencing clones from this region, we discovered that the ROR α gene was at least partially contained in BAC 28A1 and a 135-kb BAC, 287E5. ROR α is an orphan nuclear hormone receptor of the class that can bind DNA and activate transcription as a monomer in the apparent absence of ligand^{16–19}. Although ROR α has been extensively studied, its ligand, if any, remains unknown.

To study the genomic region in more detail, we used shotgun sequencing (with ninefold redundancy) to determine the nucleotide sequence of BAC 287E5. In addition, we isolated and sequenced a complementary DNA clone that contains the entire coding region for the mouse ROR α isoform. A full description of these sequences will be presented elsewhere. Comparison of the cDNA and genomic sequences revealed that 1,370 base pairs (bp) of the 1,566-bp coding region is spread over ~35 kb of BAC 287E5 and provided the complete exon–intron structure of this portion of the gene. The 5' end extends some 20 kb further, beyond BAC 28A1.

Because chimaera experiments have shown that *sg* affects Purkinje cells in a cell-autonomous fashion^{12,20}, we expected that the *sg* gene should be expressed in cerebellar Purkinje cells in normal mice and should be altered in either its expression or coding sequence in the mutant. To evaluate ROR α by these criteria (Fig. 2), we first hybridized northern blots to a probe from the hinge and ligand-binding domains of ROR α . Of three transcripts seen in wild-type cerebellum (~10.5, 6.5 and 2.4 kb), the larger two were of approximately normal length but greatly reduced abundance in *sg/sg* cerebella. Analysis using reverse

FIG. 1 Genetic and physical map of the *staggerer* locus. Top line, genetic map based on 2,497 informative meioses typed for SSLP markers and an SSCP in the *mek-1* gene. A chromosomal deficiency that removes *sg* (10FDFoD) and one that does not (99G) are shown as open bars. A minimum tiling path of YACs used as a scaffold for physical mapping and the full complement of BAC and P1 clones used for high-resolution mapping and sequencing are shown as horizontal lines. The positions of DNA polymorphisms used as genetic markers are indicated by vertical lines; meiotic recombinants are indicated by an arrowhead and the corresponding animal ID number at the bottom of the figure. Arrows indicate the genes for *ROR α* and calpactin p36 (p36), which was also found to lie nearby but outside the *sg* interval.

METHODS. Genetic mapping was performed in two crosses, B6CWD-*sg*/+ $\times$ MOLF/Ei and C57BL/6J-*sg*/+ $\times$ CAST/Ei. In the first cross, a single *sg* heterozygous male from an earlier mapping cross (C57BL/6J-*sg*/+ $\times$ CWD/Le) was mated with several MOLF/Ei females. Progeny were genotyped and *sg* heterozygotes were mated with each other as an intercross or with B6CWD-*sg*/+ partners as a backcross. In the second cross, C57BL/6J-*sg*/+ animals were mated with CAST/Ei and the resulting *sg* heterozygotes were intercrossed. Marker order was inferred by minimizing double recombinants and verified (or refined, for markers between which no recombinations were observed) by physical map STS content. STSs from the ends of YACs were isolated by inverse PCR. Additional STSs were obtained from YACs by sequencing of random *Sau*3AI and *Eco*RI subclones made from CHEF gel-isolated YAC DNA. P1 clones were obtained from Genome Systems (St Louis) using PCR assays. BAC clones (B.W.B. unpublished results) were identified by a combination of PCR assays and

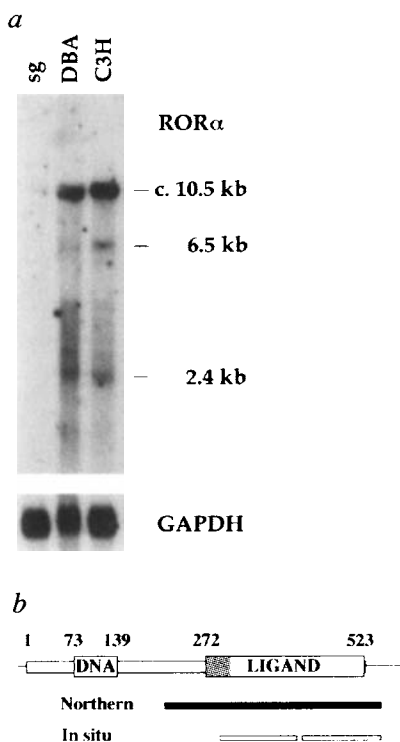


oligonucleotide hybridization. Ends of physical mapping clones were isolated by inverse PCR and sequenced to provide additional STS coverage.

FIG. 2 Evaluation of *ROR α* as a candidate gene for *staggerer*.

a, Northern blot hybridization shows that *ROR α* transcripts are expressed in adult *staggerer* cerebellum at low levels relative to wild-type controls. The blot was stripped and rehybridized with GAPDH as a control for the amount of RNA loaded in each lane. **b**, *ROR α* gene product, showing the positions of the DNA-binding domain and ligand-binding domain homology. The line indicates sequences used to probe the northern blot shown in **a**; shaded bar indicates sequence missing from *sg* transcripts. Numbered amino-acid positions refer to the mouse *ROR α* 1 isoform. **c**, Key sequence features of the region deleted in *sg*. Exons are shown in capital letters, introns in lower case. Omitted sequences are represented by ellipses. Deletion breakpoints are marked with arrows; only one copy of the underlined sequence GCTA is retained in *sg*. Wild-type coding sequence translation is shown above the nucleotide sequence of the exons, and the shifted frame translation of the predicted *staggerer* protein is given below the nucleotide sequence. Conceptual translation of the *sg* *ROR α* coding sequence from both genomic and RT-PCR sequencing predicts a frameshift at amino-acid 273 of the α 1 isoform, leading to a truncation 27 residues later. Sequence of PCR fragments from *sg*, C3H/HeJ, BALB/cJ and subclones from a 129/Sv-derived BAC demonstrates that the sequence deleted from the RNA corresponds to a single 122-bp exon removed by a 6.5-kb genomic deletion.

METHODS. RNA was isolated from frozen tissue with the Trizol reagent and polyadenylated RNAs selected by oligo(dT)-cellulose chromatography. For



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A D S A V S S F Y L D I Q P S P D Q S G
GCCGACTCAGCGTCAGCAGCTTCTACCTGGACATCCAGCCCTCCCAGACAGTCGCGGA

L D I N G I K P E P I C D Y T P A S G F
TTGGACATCAATGGGATCAAAACCGAAGCCATATGTGACTACACACAGCATCTGGCTTC

F P Y C S F T N G E T S P T V S M A E L
TTCCCTACTGTCTCTTCAACAGGAGAGACTTCCCAACCGTGTCATGGCAGAACTA

E
Ggttaaggcagtagtcagtgctctcctcctgg.....gccttaccacagcacatg
A
gtatttttg|gctaacagtgacacaaatccatgactagggtc.....tgatct

          H L A Q N I S K S H L E T C
cctgtttttctcccccagAACACCTTGCCAGAACATATCCAAATCCCACCTGGAACCTGC

Q Y L R E E L Q Q I T W Q T F L Q E E I
CAGTACTTGGCGGAAGAGCTCCAGCAGATAACGTGGCAGACCTTCTTCGAGGAGAGATT

E N Y Q N K
GAAACTACCAGAACAAGgttagagttgaggactttctcttccca.....

ctccatttccatcaccttcaaaactgtaa|gctaggaatctcagtaggcagaaaggatcgg

aagcaggctttttataacctaagtaacagagactattttttttaacctataatctttttt

taaccattttccaaagaaagctttttacaaactaggaagccagcccgaggctggtgcac

          Q R E V M
cagggttccagctagttcttatgctaagtgtgcttctcttccacagCAGAGAGAGGTGA
          E R G D

W Q L C A I K I T E A I Q Y V V E F A K
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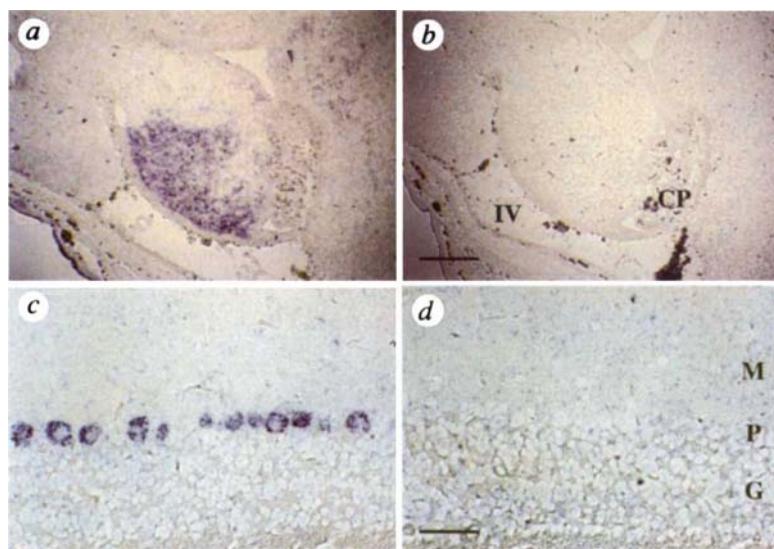
R I D G F M E L C Q N D Q I V L L K A
AACGCATGTGGATTATGTGAGCTGTGTCAAAATGATCAAAATGTGCTTCTAAAGCAG
T H *

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northern blots, 3 μ g poly(A)⁺ RNA was treated with glyoxal, electrophoresed through 1% agarose gels and transferred to nylon membranes as described³⁰. For RT-PCR, 1 μ g poly(A)⁺ RNA was reverse-transcribed and 1:100 of the resulting cDNA was used for each amplification. For sequencing, PCR products were reamplified using chimaeric primers carrying M13 sequencing primer sites and gel-isolated before cycle sequencing with fluorescently labelled primers.

FIG. 3 *In situ* hybridization of ROR α to sagittal sections of E15 mouse embryos and coronal sections through adult mouse hindbrain. The positions of the probe sequences within the ROR α gene are shown in Fig. 2b. a, c, ROR α antisense probe; b, d, sense probe. a, b, Hybridization signals detected in E15 cerebellum. The fourth ventricle (IV) and choroid plexus (CP), which lie below and posterior to the cerebellum, are marked for orientation. Anterior is to the left. Bar, 200 μ m. c, d, Hybridization to the Purkinje cell layer detected in coronal sections through adult cerebellum. Letters denote the molecular (M), Purkinje cell (P) and granule cell (G) layers. Bar, 50 μ m.

METHODS. Two cDNA fragments $\sim$ 500 bp were used for each sense and antisense probe. cDNA corresponding to the ROR α constant region were amplified by RT-PCR (primers 6680U.2: AGTTGGTCGGATGCTCAAG and rora.2: GGCTGCAGAAATGCCTGG), reamplified with internal chimaeric primers bearing priming sites for dye-primer sequencing, gel-purified and sequenced. Sequenced fragments were reamplified with chimaeric primers, either with the antisense strand carrying a T7 RNA polymerase promoter (T7rora.8: TGTAATACGACTCACTATAGGGCGAGTCAAAGGCACGGCAC or T7rora.6: TGTAATACGACTCACTATAGGGCGATCTAGAAGTGCTCGG-GCG), or with the sense strand carrying a T3 RNA polymerase promoter (T3rora.3: AATTAACCCTCACTAAAGGGCGACTCAGCCGTCAGCAG or T3rora.7: AATTAACCCTCACTAAAGGGCATTTGACGGGAAGTATGCG), and gel-isolated. RNA probes incorporating 11-digoxigenin-UTP were synthesized with the appropriate polymerase.



Hybrid-ready tissue sections (from Novagen) were processed according to the protocol of A. Nieto (Mill Hill), and visualized with an alkaline-phosphatase-conjugated anti-digoxigenin antibody using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium substrates.

transcription and the polymerase chain reaction (RT-PCR) revealed a 122-bp deletion, which removes the start of the ligand-binding homology domain and shifts the reading frame, causing a stop codon after 27 amino acids. Comparison with the genomic sequence showed that the deletion corresponds to a single exon.

Southern blot and long-range PCR analysis revealed a 6.5-kb genomic deletion relative to several wild-type chromosomes. The precise deletion breakpoints were localized by sequencing genomic PCR products (Fig. 2). The deletion is not present on C3H/HeJ chromosomes, on which *sg* apparently arose. (Specifically, *sg* arose in F₂ progeny of a non-inbred V-*ob/ob* male and a C3H/He $\times$ BALB/cHm female (P. W. Lane, personal communication). Of existing strains related to these progenitors, the *sg* chromosome most closely resembles C3H/HeJ, having identical allele sizes at 16 SSLPs within 1 cM of *sg*.)

The deletion would be predicted to create a null or severe hypomorphic allele, because truncation of the ligand-binding homology domain of ROR α has been shown to preserve DNA binding but greatly to reduce or abolish transactivation activity of the unliganded receptor *in vitro*¹⁹. The predicted loss-of-function is consistent with our observation that *sg* behaves genetically as a null allele, with the behavioural and histological phenotype in *sg*/Df 10FDFoD mice being no more severe than in C57BL/6J-*sg/sg*.

We examined the pattern of ROR α RNA expression in mouse embryos and adult brain to determine whether it is consistent with the cell-autonomous Purkinje cell defects seen in young homozygotes and the accelerated atrophy seen in heterozygotes (Fig. 3). Purkinje neurons arise from a proliferative zone above the fourth ventricle beginning on embryonic day 13 (E13) in normal mice and migrate along radial glia from E14 to E17; they form a laminar shell in the presumptive cerebellar cortex and position themselves in a monolayer during postnatal development²¹. *In situ* hybridization to sagittally sectioned embryos reveals prominent expression in large cells of the cerebellar anlage by E14. The position and size of the ROR α -positive cells in embryonic cerebellum is consistent with Purkinje cell precursors. In addition, hybridization to coronal sections through adult hindbrain reveals expression only in the Purkinje cell layer. Prominent hybridization is also detected in embryonic midbrain, with weaker signals in thymus, whisker follicles, eye, lung, kidney tubules, gut. The expression in thymus is particularly interesting as *sg/sg* mice have been reported

to have delayed thymic development and a defect in terminating T-cell responses²².

The data provide compelling evidence that the deletion in ROR α is the cause of the *sg* defect: the deletion lies in the small (162-kb) region containing *sg*, is not found on the presumed ancestral chromosome (or in 20 other strains examined) and eliminates a domain required for transcriptional activity *in vitro*, and the gene is expressed in Purkinje cells at the expected time in development. Ultimate proof will require transgenic rescue.

Identification of the *sg* gene as a nuclear hormone receptor is particularly informative given the key role that thyroid hormone (TH) plays in cerebellar development. Hypothyroidism causes reduced dendritic arborization of Purkinje cells and decreased granule-cell proliferation similar to that seen in *sg* mutants, whereas TH replacement alleviates these defects in a dose-dependent fashion²³. Interestingly, the *sg* mutation blocks Purkinje cell response to TH²⁴. One apparently direct target of TH action is the Purkinje cell protein-2 (*pcp-2*) gene, which responds to TH levels *in vivo* and which is activated by ligand-bound TH receptor- β (TR β) in transfection assays²⁵. Accordingly, we used RT-PCR to test whether *sg* affects expression of either TR β or *pcp-2* RNA. We found that *pcp-2* expression is undetectable in *sg* mutants, despite significant levels of TR β expression (not shown). Taken together, this strongly suggests that ROR α interacts with a thyroid-hormone signalling pathway in the maturation of cerebellar Purkinje cells. Such a model would be consistent with recent examples of crosstalk among nuclear hormone receptor signalling pathways^{26–29}. Further elucidation of the ROR α signalling pathway should provide new insights into these regulatory interactions and the morphological, synaptogenic and trophic events that are defective in *staggerer*. □

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Characterization of a cancer cachectic factor

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CANCER cachexia is a syndrome of progressive wasting which has been suggested to be mediated by tumour-necrosis factor- α (ref. 1), interleukins 1 and 6 (ref. 2), interferon- γ (ref. 3) and leukaemia-inhibitory factor⁴. It has proved difficult to correlate levels of tumour-necrosis factor- α and interleukin-6 with cancer cachexia^{5,6}, and the weight loss induced by leukaemia-inhibitory factor may be due to toxicity⁷. In the murine adenocarcinoma MAC16, cachexia is mediated by circulatory catabolic factors^{8,9}, which we have now isolated using an antibody cloned from splenocytes of mice transplanted with the MAC16 tumour, with a delayed cachexia¹⁰. The material is a proteoglycan of relative molecular mass 24K which produces cachexia *in vivo* by inducing catabolism of skeletal muscle. The 24K material was also present in urine of cachectic cancer patients, but was absent from normal subjects, patients with weight loss due to trauma, and cancer patients with little or no weight loss. This suggests that cachexia in mice and humans may be produced by the same material.

Antigenic material from the MAC16 tumour was purified by affinity chromatography with the mouse monoclonal antibody and subsequent reverse-phase hydrophobic chromatography (Table 1). Immunoreactive material from the affinity column consisted of two electrophoretic species of relative molecular mass (M_r) 69K and 24K (Fig. 1a), both of which contained carbohydrate, as determined with a digoxigenin glycan detection kit. These two fractions were resolved by reverse-phase chromatography (Fig. 1b) and the 24K species was found to be present as a

single band by silver staining of SDS-polyacrylamide gels (Fig. 1c). The amino-terminal sequence of this material was determined as: Tyr-Asp-Pro-Glu-Ala-Ala-Ser-Ala-Pro-Gly-Ser-Gly-Asp-Pro-Ser-His-Glu-Ala-(Ser)-(Ala). This sequence showed no homology with recognized cytokines or other eukaryotic proteins. There is some homology with a streptococcal preabsorbing antigen

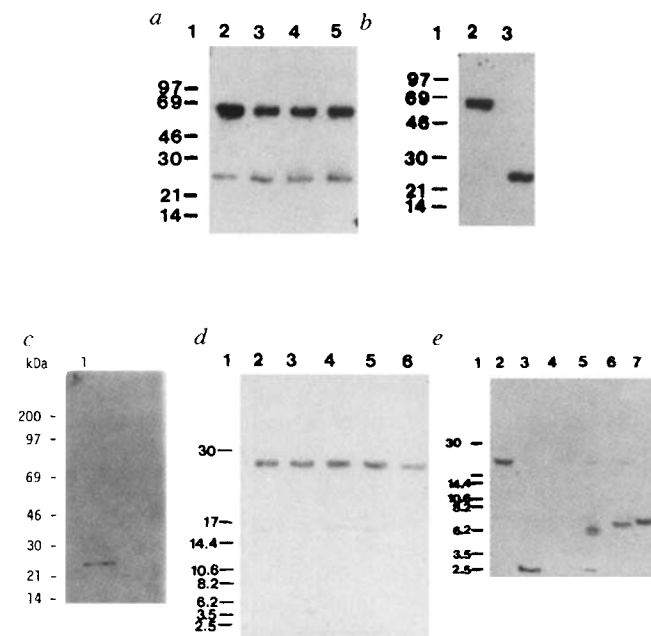


FIG. 1 a, Western blot of immunoreactive fractions (lanes 2–5 representing the band of ELISA reactivity) isolated by affinity chromatography of a MAC16 tumour homogenate fractionated on 15% SDS-polyacrylamide gels and detected with the mouse monoclonal antibody. Lane 1 contains M_r standards. b, Immunoblot of ELISA-positive fractions eluted from the C_8 column. Lane 1, M_r markers, labelled in thousands; lanes 2 and 3, material eluting at 81% and 55% acetonitrile. c, Silver stain of SDS gel of material eluting at 55% acetonitrile. d, Autoradiographs of iodinated material after electrophoresis on 16.5% SDS-polyacrylamide gels after treatment with proteolytic enzymes for 1 h at 37 °C. Lane 1, M_r markers; lane 2, no treatment; lane 3, pronase (0.43 U) in 100 mM Tris-HCl (pH 8.0); lane 4, chymotrypsin (30 μ g); lane 5, trypsin (30 μ g); and lane 6, pepsin (20 μ g) in 100 mM Tris-HCl (pH 8.0) and 2 mM CaCl_2 . e, Treatment to remove carbohydrate from the iodinated material. Lane 1, M_r markers; lane 2, no treatment; lane 3, chemical deglycosylation with a Glycofree deglycosylation kit; lane 4, treatment with 2 M NaBH_4 in 0.1 M NaOH at 37 °C for 16 h; lane 5, treatment with peptide-N-glycosidase F (1 U, 0.05 μ g protein recombinant), endo- α -N-acetylglucosaminidase (1 mU, 40 μ g protein) and sulphatase (1.6 U); lane 6 with peptide-N-glycosidase F, endo- α -N-acetylglucosaminidase and neuraminidase (0.12 mU); or lane 7 with peptide-N-glycosidase F and endo- α -N-acetylglucosaminidase. A mock-treated control showed no change in M_r .

METHODS. Hybridomas were produced by fusion of splenocytes from mice transplanted with the MAC16 tumour with mouse BALB/c myeloma cells using polyethylene glycol 1500. Clones were screened against purified material of M_r 24K (ref. 10). The antibody-producing cells were single-cell cloned by limiting dilution and the process was repeated three times. Monoclonal antibody was purified using a column of protein-A linked to Sepharose CL4B. Fractionated proteins were transferred to nitrocellulose membranes that had been blocked with 5% Marvel, incubated with the monoclonal antibody followed by a protein-A-peroxidase conjugate. Bands were detected with an ECL system. Material eluting at 55% acetonitrile was dialysed against water in a final volume of 200 μ l and iodinated using Na^{125}I (0.1 mCi, specific activity 17.4 mCi μ g⁻¹) and iodobeads. Isolated gastrocnemius muscle was suspended in 4 ml RPMI 1640 medium without phenol red in the presence of 9 nM labelled 24K material and incubated for 30 min at 37 °C under an atmosphere of O_2 and CO_2 (19:1), and the radioactivity remaining bound after extensive washing was determined. For protein degradation after the preincubation with 24K material (6 nM), tyrosine release was determined by a fluorometric method as described³.

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involved in the pathogenesis of acute post-streptococcal glomerulonephritis¹¹, although this antigen showed no crossreactivity with the MAC16 monoclonal antibody and the tumour was free of any microbiological contamination. The 69K species gave the same N-terminal sequence together with that for mouse albumin. The 24K material could also be isolated from MAC16 cells *in vitro* (Table 1). The tumour origin has not been confirmed by Southern or northern blotting because of the high degree of degeneracy of the codons for the terminal seven amino acids.

Attempts to obtain further sequence information by proteolysis of the 24K material were unsuccessful, suggesting either resistance to proteolysis or that the original sequence was complete. To investigate the structure, the N-terminal tyrosine was iodinated using Na¹²⁵I and the labelled material was incubated with a range of proteolytic enzymes. The *M_r* of the iodinated material was unaffected by treatment with pronase, chymotrypsin, trypsin or pepsin (Fig. 1d).

Both peptide-N-glycosidase F (PNGase F) and endo- α -N-acetylgalactosaminidase (O-glycanase) gave 14K fragments, whereas a combination of the two enzymes with or without neuraminidase gave a 6K fragment (Fig. 1e). A 14K fragment was also generated after treatment with chondroitinase AC. Enzyme treatment destroyed antibody reactivity. These results suggest the presence of both N- and O-glycan chains, including glycosaminoglycans with sulphate residues. Chemical deglycosylation of the iodinated material with anhydrous trifluoromethanesulphonic acid pro-

duced a single band at 2K, a size close to the calculated mass of the sequenced peptide (1.9K). Thus the sequence obtained represents the whole or most of the polypeptide portion of the molecule.

Intravenous administration of either affinity-purified material (30 μ g protein) (Fig. 2a) or the single 24K species (30 ng protein) (Fig. 2b) produced a rapid weight loss which could be prevented by prior administration of the monoclonal antibody (Fig. 2a). In contrast, neither the peptide portion of the molecule nor the non-immunogenic fractions from the reverse-phase column produced a change in body weight (Fig. 2b). Food (3.3 g per mouse) and water (2.8 ml per mouse) intake over the 24-h period were not reduced (values for controls were 2.5 g per mouse and 2.8 ml per mouse, respectively), suggesting that the material was not toxic. Body composition analysis⁹ revealed a 29% loss of adipose tissue and a 14% loss of carcass dry weight ($P = 0.05$), with no change in total body water content. Blood glucose levels were significantly decreased ($P = 0.01$). There was no alteration in plasma triglyceride levels, unlike the effect observed with cytokines¹⁻³. The action of the purified material in mice was similar to that produced by transplantation of the MAC16 tumour in that: (1) weight loss occurred without a reduction in food and water intake⁸, possibly due to an increased energy expenditure¹²; (2) loss of adipose tissue exceeded loss of lean body mass¹²; and (3) there was marked hypoglycaemia¹⁴. Body-composition changes and blood glucose levels remained at control values with prior administration of the

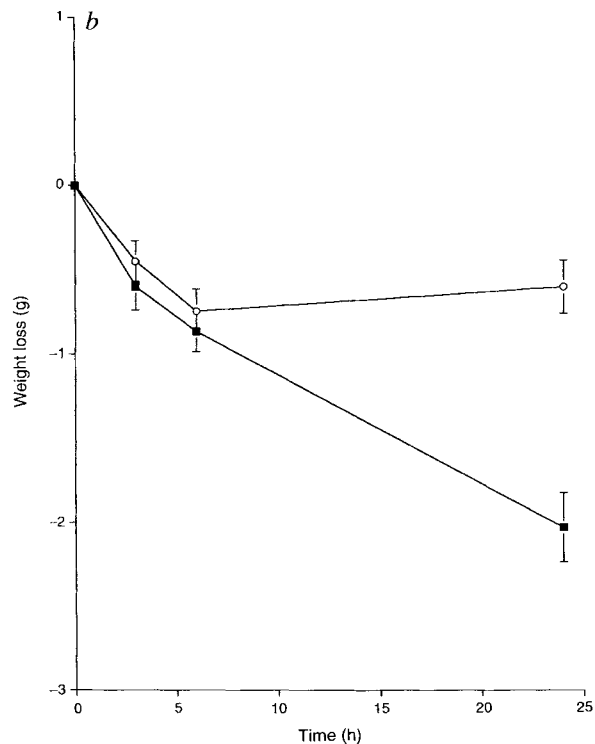
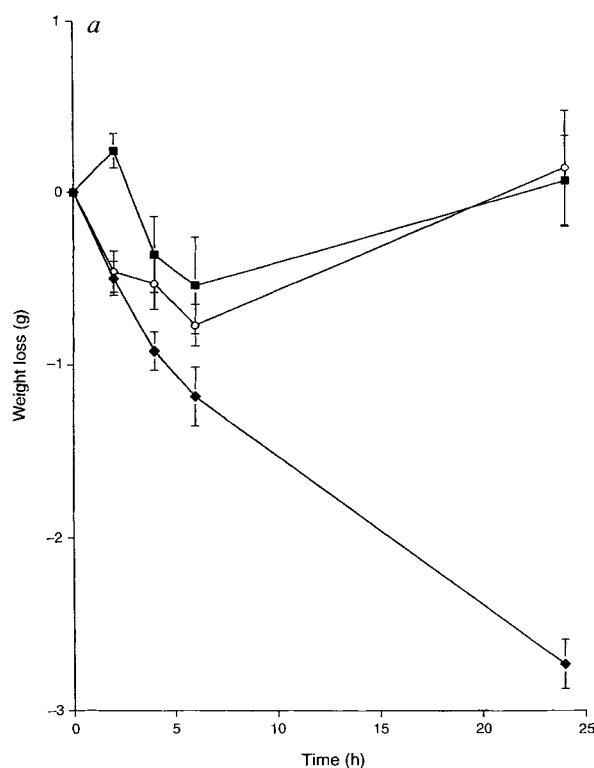


FIG. 2 a, Effect of affinity-purified MAC16 tumour extract on body weight of female NMRI mice. Immunoreactive material was concentrated with an Amicon filtration cell (molecular size cut-off, 3 K) against Ca²⁺/Mg²⁺-free phosphate-buffered saline (PBS). The concentrate was resuspended in PBS (8.3A₄₉₂ units ml⁻¹) and portions (150 μ l; 7 μ g protein) were injected into the tail vein of five female NMRI mice at 1.5-h intervals (10:30, 12:00, 13:30 and 15:00) (◆). Animals were weighed before each injection with the final determination at 24 h. Control animals received PBS by tail vein injection (○), while a third group received monoclonal antibody (0.8 mg protein in 350 μ l PBS by i.p. injection) 24 h before the first injection of the affinity-purified material (■). Both food and water intake were monitored. The results are the mean $\pm$ s.e.m. for 5 mice per group and the experiment has been repeated three times. b, Change in body weight of female NMRI

mice after intravenous administration of material eluting at 55% acetonitrile (■) (2.1A₄₉₂ units; 30 ng protein per mouse) or 87% acetonitrile (○) (5 μ g protein per mouse) from the C₈ column. After the addition of PBS (3 ml), fractions were placed under a steady stream of nitrogen for 40 min to remove the acetonitrile. Fractions were concentrated against PBS using an Amicon filtration cell (molecular size cut-off at 10K). Portions (150 μ l) were injected into the tail vein of five mice at 1.5-h intervals. Animals were weighed as in a and the results are the mean $\pm$ s.e.m. for 6 mice per group. For the measurement of protein degradation *in vivo*, mice were labelled with L-[4-³H]phenylalanine and the release of radioactivity from gastrocnemius muscle was determined after 24 h as described¹⁷. Animals treated with the synthetic peptide (4 $\times$ 7 μ g) showed no weight change compared to paired controls.

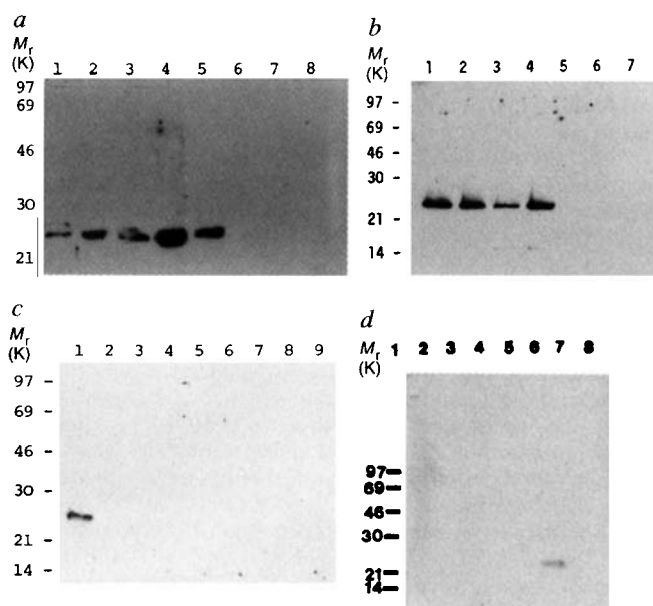


FIG. 3 *a*, Immunoblot of unfractionated urine obtained from pancreatic cancer patients (lanes 1 to 5) and normal subjects (lanes 6 to 8) using the MAC16 mouse monoclonal antibody which was biotinylated and detected with an ECL system (Amersham, UK). Urine (20 ml) was treated with $(\text{NH}_4)_2\text{SO}_4$ (80%) overnight at 4 °C. The precipitated protein was recovered by centrifugation at 3,000g for 30 min, dialysed against water, and concentrated. Each lane contains 15 μg protein. All cancer patients had a weight loss > 1.5 kg per month and had UICC staging: lane 1, stage II; lanes 2 and 3, stage III; lane 4, stage III/IV; and lane 5, stage IV carcinoma of the pancreas. Lanes 6–8, urine from normal subjects. *b*, Immunoblot of 80% $(\text{NH}_4)_2\text{SO}_4$ -precipitated urine from patients with pancreatic cancer: lane 1, stage IV weight loss 2 kg per month; lane 2, stage III weight loss 3 kg per month; lane 3, stage IV weight loss 2.5 kg per month; lane 4, stage III weight loss 2.7 kg per month; lane 5, stage IV no weight loss; lane 6, stage III weight loss 0.13 kg per month; lane 7, stage IV weight loss 0.68 kg per month. *c*, Immunoblot of $(\text{NH}_4)_2\text{SO}_4$ -precipitated urine from patients with: lane 1, pancreatic cancer, stage III, weight loss 1.5 kg per month; lanes 2 and 3 from patients with major burns; lane 4, catabolism and sepsis; lane 5, ulcer; and lanes 6–9, multiple injuries. *d*, Immunoblot of material extracted from the urine of a patient with pancreatic carcinoma and cachexia, purified as for Table 1. The lanes contain fractions eluted from the reverse-phase C8 column, identified by absorption at 214 nm. Lane 1, M_r markers; lanes 2 to 8, decreasing concentrations of acetonitrile: lane 2, 81%; lane 3, 76%; lane 4, 69%; lane 5, 65%; lane 6, 59%; lane 7, 56%; lane 8, 54%.

monoclonal antibody. These results indicate that the purified material can induce cachexia. In addition, the material caused an enhanced protein degradation from isolated gastrocnemius muscle, as measured by tyrosine release¹³ (50 μmol tyrosine released per g muscle over a 1.5-h period with 6 nM material), which was destroyed by incubation with PNGase F or *O*-glycosidase. Neither tumour-necrosis factor- α (ref. 15) or interleukin 6 (ref. 16) are capable of initiating direct muscle catabolism *in vitro*. When incubated with 9 nM labelled material, isolated muscle strongly bound 2.3 fmol g⁻¹ under the same conditions as those used for tyrosine release (Fig. 1), although it has not been determined whether this is due to a specific receptor interaction or to a lectin effect. *In vivo* studies using [4-³H]phenylalanine showed a 60% increase in protein degradation and a 50% decrease in protein synthesis in gastrocnemius muscle.

To determine whether this cachectic factor was associated with human cancer, we probed for immunoreactive material in the urine of 16 normal subjects, 17 subjects with weight loss due to trauma or sepsis, and 25 cancer patients. Of the cancer patients, 18 had pancreatic carcinoma and, if the weight loss was > 1.5 kg per

month, showed the 24K material on immunoblots (Fig. 3*a*). This material was not detectable in the urine of normal subjects, nor if the weight loss was < 1.3 kg per month (Fig. 3*b*). The 24K material was also not detectable in the urine of patients with weight loss due to major burns, multiple injuries or surgery-associated catabolism and sepsis (Fig. 3*c*). The ability to detect the 24K material in the urine of pancreatic cancer patients was more dependent on the rate of weight loss than the stage of the disease. The remaining cancer patients showing a weight loss of > 1.5 kg per month had carcinoma of the lung (1), breast (1) or ovary (1), or T-cell lymphoma (1), and all showed the 24K material. Patients with carcinomas of the breast (2) or lung (1) who were not actively losing weight also showed no evidence of the 24K material, suggesting that it is specific to the cachectic state and appears not to be confined to a particular type of cancer.

Urine from a patient with pancreatic carcinoma and weight loss was fractionated according to the scheme used for the mouse tumour (Table 1). An immunoreactive species eluted at 56% acetonitrile from the reverse-phase column, and this produced a single 24K band on immunoblots developed with the mouse

TABLE 1 Purification of cachexia-inducing material from the MAC16 tumour

Stage	Protein (mg)	Protein recovery (%)	A ₂₁₄ Units	Recovery (%)	Specific activity units (mg ⁻¹)	Purification (fold)
Tumour homogenate	3,160	—	—	—	—	—
40% $(\text{NH}_4)_2\text{SO}_4$	113	5.3	—	—	—	—
Affinity column	0.256	0.012	43*	—	168	—
C ₈ HPLC						
55% CH ₃ CN	1.2×10^{-4}	3.8×10^{-6}	8.6	29	71,667	427
81% CH ₃ CN	0.124	4×10^{-3}	3.8	9	31	0.18

Solid tumours obtained from mice with cachexia were homogenized in 10 mM Tris-HCl (pH 8.0) containing 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 0.5 mM EGTA, and 1 mM dithiothreitol at a concentration of 5 ml g⁻¹ tumour. Debris was removed by low-speed centrifugation, the supernatant was diluted and $(\text{NH}_4)_2\text{SO}_4$ (40% w/v) was added. The supernatant was concentrated by ultrafiltration and applied to an affinity column containing monoclonal antibody (see legend to Fig. 1) coupled to Affi Gel Hz (Bio-Rad, Hemel Hempstead, UK). The column was washed with 10 mM Tris-HCl (pH 8.0) and the retained proteins were eluted with 100 mM glycine-HCl (pH 2.5). The immunoreactive fractions determined by an enzyme-linked immunoassay (ELISA) plate assay were pooled and concentrated against water using an Amicon filtration cell until a protein concentration of 2 mg ml⁻¹ was achieved. Portions (50 μl) were fractionated using a Brownlee Aquapore RP-300, C₈ column (Applied Biosystems Ltd) size 100 $\times$ 2.1 mm. The flow rate was 0.2 ml min⁻¹ and the mobile phase was HPLC-grade water (Fisons, Loughborough, UK) containing 0.06% trifluoroacetic acid (TFA) and acetonitrile 190 (Rohm Chemicals Ltd, Loughborough, UK) containing 0.04% TFA with a gradient of 2 to 65% acetonitrile in water over a 30 min period, followed by 65 to 100% acetonitrile in water over 10 min and 100% acetonitrile for 10 min. Absorbance at 214 nm was monitored and peaks were collected into microfuge tubes. Immunologically identical material could be isolated from MAC16 cells *in vitro*.

* Because of reaction of protein-A with mouse immunoglobulins in the solid tumour, specific immunoreactivity towards the mouse monoclonal antibody was not determined before the affinity chromatography.

monoclonal antibody (Fig. 3d). The amino-acid sequence analysis (up to residue 14) was identical to that for the mouse. Thus, cachexia in mice and humans appears to be associated with the same material.

These data suggest that the 24K proteoglycan represents a potential mediator of the process of cancer cachexia. It is distinguished from the cytokines, not only in structure, but by the ability to accelerate breakdown of skeletal muscle *in vitro* and *in vivo* and to produce weight loss *in vivo* by a process not involving anorexia. Thus this material may induce the metabolic component of cachexia, because the cytokines are invariably associated with anorexia. The 24K proteoglycan shows a strong association with the development of clinical cancer cachexia, because immunoreactive material is detectable in the urine of all patients so far investigated who are actively losing weight, irrespective of tumour type. This suggests that this material may play an active role in the development of human cancer cachexia. □

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Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family

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A GENERAL property of signal transduction pathways is that prolonged stimulation decreases responsiveness, a phenomenon termed desensitization. Yeast cells stimulated with mating pheromone activate a heterotrimeric G-protein-linked, MAP-kinase-dependent signalling pathway that induces G1-phase cell-cycle arrest and morphological differentiation (reviewed in refs 1, 2). Eventually the cells desensitize to pheromone and resume growth³. Genetic studies have demonstrated the relative importance of a desensitization mechanism that uses the *SST2* gene product, Sst2p^{4–7}. Here we identify a mammalian gene family termed *RGS* (for regulator of G-protein signalling) that encodes structural and functional homologues of Sst2p. Introduction of *RGS* family members into yeast blunts signal transduction through the pheromone-response pathway. Like *SST2* (refs 8–10),

they negatively regulate this pathway at a point upstream or at the level of the G protein. The *RGS* family members also markedly impair MAP kinase activation by mammalian G-protein-linked receptors, indicating the existence and importance of an *SST2*-like desensitization mechanism in mammalian cells.

Although close homologues of Sst2p have not been reported, we studied an expanding family of mammalian genes that encodes proteins with a conserved domain (RGS domain) related to a region in Sst2p. It is even more closely to a region in flbA, a protein encoded by an *SST2*-related gene in *Aspergillus nidulans*¹¹. The first *RGS* family member, *BL34* (*RGS1*), was found during screens for B-lymphocyte-specific genes and activation genes in chronic lymphocytic leukaemia cells^{12,13}. A search for activation genes in peripheral blood mononuclear cells, which led to the isolation of *GOS8* (ref. 14), provided evidence that *RGS1* belongs to a gene family. The predicted *GOS8* (*RGS2*) and *RGS1* proteins have similar relative molecular mass and 42% amino-acid identity. In addition, an evolutionarily conserved homologue was found in *Caenorhabditis elegans*¹⁵.

A Southern blot, using human genomic DNA hybridized with an oligonucleotide that was based on a highly conserved portion of the RGS domain, predicted a minimum of 15 family members (data not shown). A screen of a B-cell complementary DNA library with the same probe identified a new *RGS* family member, *RGS3*, which encodes a protein of 519 amino acids and has a relative molecular mass of 57,000 (*M_r* 57K) (Fig. 1a,b). An acidic region (residues 181–226), predicted to assume a coiled-coil¹⁶, is located outside the region homologous to *RGS1* and *RGS2*. To the amino-terminal side of the coiled-coil is a region rich in proline and glutamine that contains a hexapeptide repeat of the motif (Q,K)(D,E)(P,L)(P,L)(P,X)X and overlaps four PEST sequences (amino acids 30–46, 48–64, 66–82 and 84–111). Northern blot analysis (Fig. 1c) indicated that there are two major *RGS3* transcripts present in multiple tissues.

The identification of a fourth *RGS* family member in a screen of rat brain cDNAs, the expression of which allowed *sst2Δ* mutants to grow on pheromone-containing plates, provided the first direct evidence of a functional significance of the sequence homologies noted between the *RGS* family members and *SST2*. *RGS4* encodes a protein of 205 amino acids, 37% identical to *RGS1* (Fig. 2a). A TFasta database search identified a partial cDNA sequence (GBN:HSC1YC111, accession number F07191) for the human orthologue, and a human *RGS4* cDNA (the protein of which has 97% amino acid identity with rat *RGS4*) was isolated. Further database searches identified three other putative *RGS* family members: one from infant brain and lung (accession numbers D311257 and R35272); a second from fetal lung and infant brain (accession numbers H09621 and L40394); and a third from adult lung (accession number T94013). These three genes were designated *RGS5*, *RGS6* and *RGS13*, respectively (Fig. 2b). Northern blot analysis identified *RGS4* transcripts only in brain (Fig. 2c).

To determine whether other *RGS* family members function in yeast, we introduced expression plasmids for *RGS1*, *RGS2*, *RGS3* and *RGS4*, as well as a truncation of *RGS3* (*RGS3T*) that retains the coding region for the RGS domain (residues 314–519), into an *sst2Δ* strain. Their expression partially complemented the pheromone-supersensitive phenotype of the *sst2Δ* mutant, as judged by growth-arrest (halo) assays. Dose-response relationships indicated that they reduced pheromone sensitivity as follows: *RGS4*, >30-fold; *RGS1* and *RGS3T*, 10–30-fold; *RGS3* and *RGS2*, 3-fold (Fig. 3a). Furthermore, their expression in wild-type cells decreased pheromone sensitivity, as indicated by the formation of turbid zones of growth inhibition (Fig. 3b), and expression of *RGS4* in the *sst2Δ* mutant partly blocked pheromone-induced expression of a reporter gene, both in terms of apparent EC₅₀ (the effector concentration for half-maximum response) and maximal level of expression (Fig. 3c).

We used genetic tests to determine where in the pheromone response pathway the *RGS* family members function. As judged by assays of G1 arrest and transcriptional responses, neither *RGS4*

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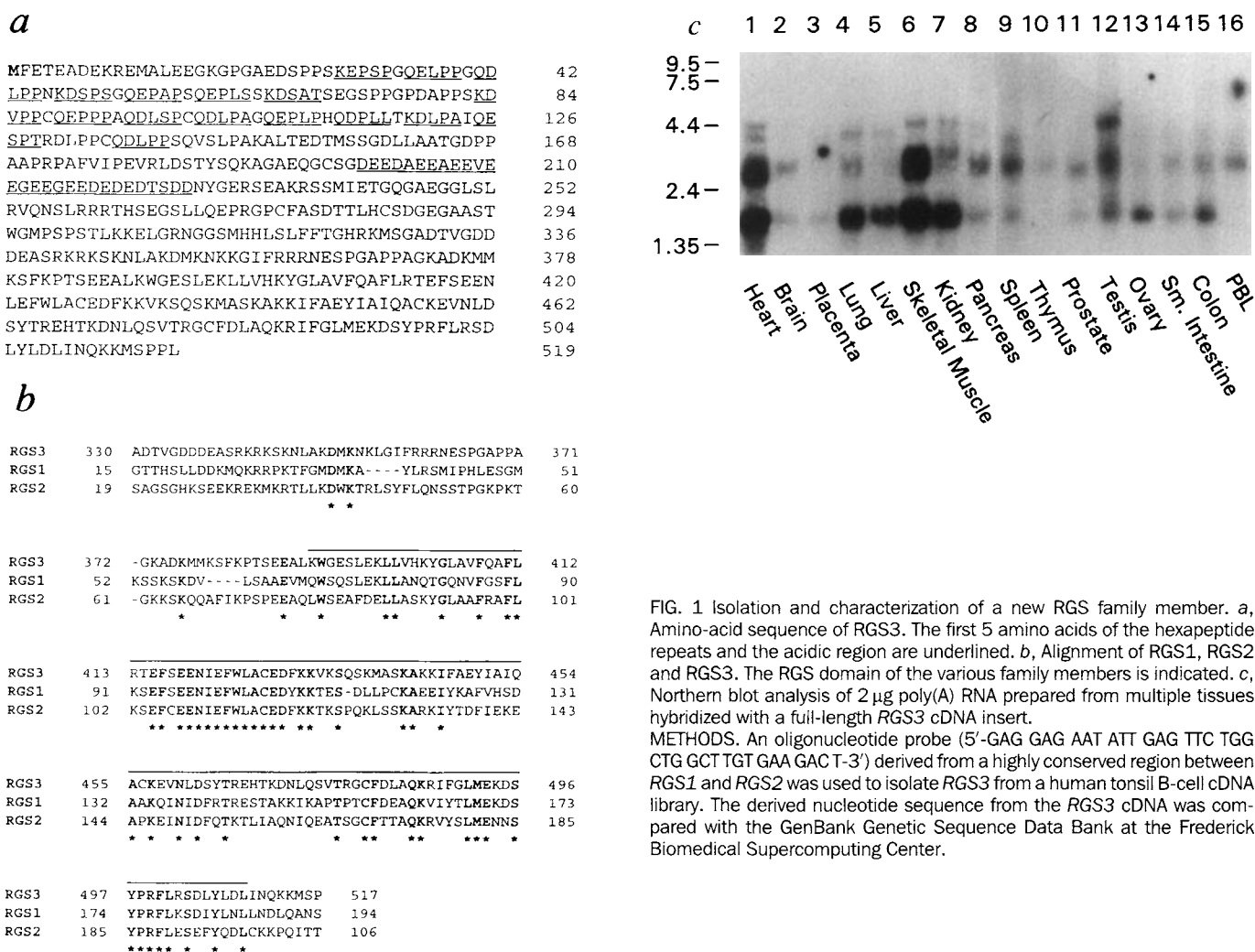


FIG. 1 Isolation and characterization of a new RGS family member. **a**, Amino-acid sequence of RGS3. The first 5 amino acids of the hexapeptide repeats and the acidic region are underlined. **b**, Alignment of RGS1, RGS2 and RGS3. The RGS domain of the various family members is indicated. **c**, Northern blot analysis of 2 µg poly(A) RNA prepared from multiple tissues hybridized with a full-length RGS3 cDNA insert.

METHODS. An oligonucleotide probe (5'-GAG GAG AAT ATT GAG TTC TGG CTG GCT TGT GAA GAC T-3') derived from a highly conserved region between RGS1 and RGS2 was used to isolate RGS3 from a human tonsil B-cell cDNA library. The derived nucleotide sequence from the RGS3 cDNA was compared with the GenBank Genetic Sequence Data Bank at the Frederick Biomedical Supercomputing Center.

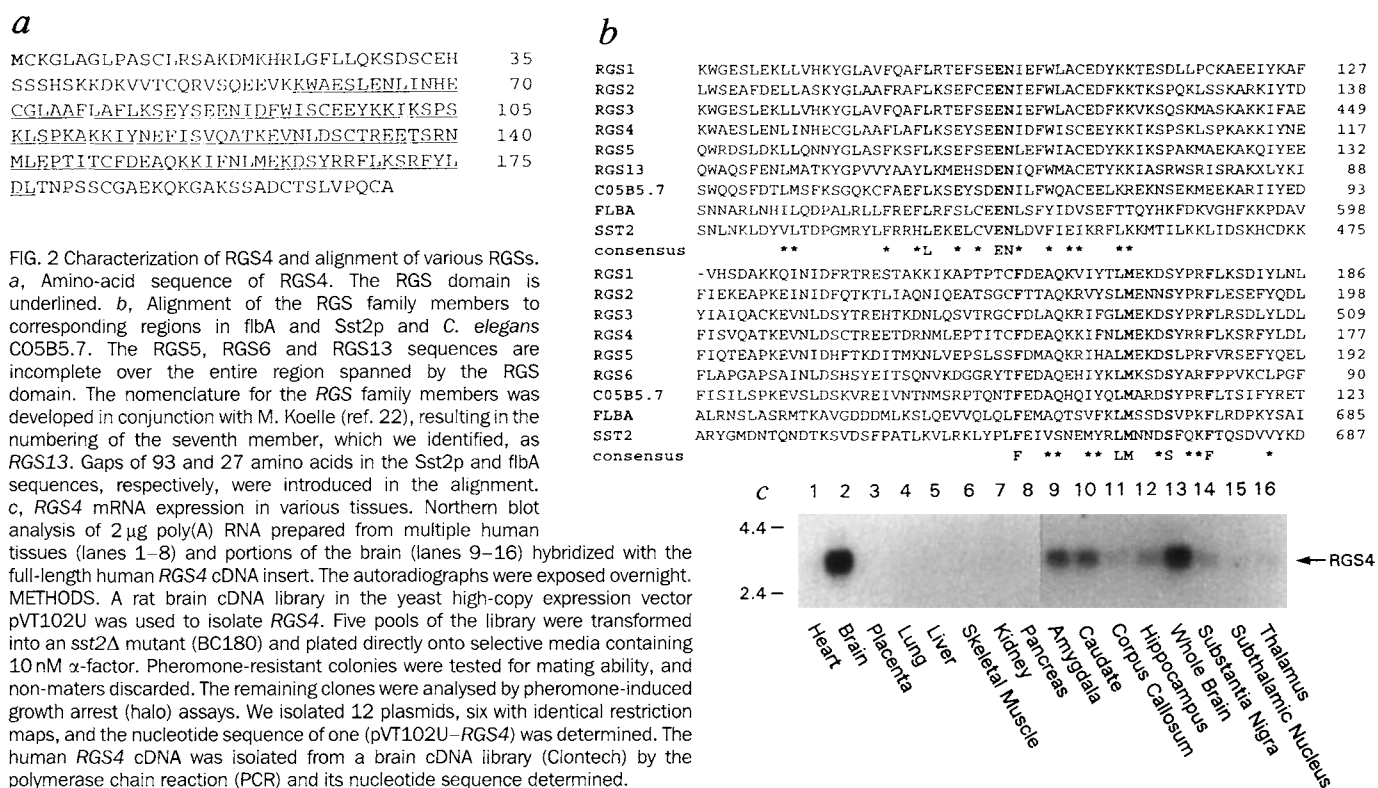


FIG. 2 Characterization of RGS4 and alignment of various RGSs. **a**, Amino-acid sequence of RGS4. The RGS domain is underlined. **b**, Alignment of the RGS family members to corresponding regions in flbA and Sst2p and *C. elegans* C05B5.7. The RGS5, RGS6 and RGS13 sequences are incomplete over the entire region spanned by the RGS domain. The nomenclature for the RGS family members was developed in conjunction with M. Koelle (ref. 22), resulting in the numbering of the seventh member, which we identified, as RGS13. Gaps of 93 and 27 amino acids in the Sst2p and flbA sequences, respectively, were introduced in the alignment. **c**, RGS4 mRNA expression in various tissues. Northern blot analysis of 2 µg poly(A) RNA prepared from multiple human tissues (lanes 1-8) and portions of the brain (lanes 9-16) hybridized with the full-length human RGS4 cDNA insert. The autoradiographs were exposed overnight. **METHODS.** A rat brain cDNA library in the yeast high-copy expression vector pVT102U was used to isolate RGS4. Five pools of the library were transformed into an *ssd2Δ* mutant (BC180) and plated directly onto selective media containing 10 nM α -factor. Pheromone-resistant colonies were tested for mating ability, and non-maters discarded. The remaining clones were analysed by pheromone-induced growth arrest (halo) assays. We isolated 12 plasmids, six with identical restriction maps, and the nucleotide sequence of one (pVT102U-RGS4) was determined. The human RGS4 cDNA was isolated from a brain cDNA library (Clontech) by the polymerase chain reaction (PCR) and its nucleotide sequence determined.

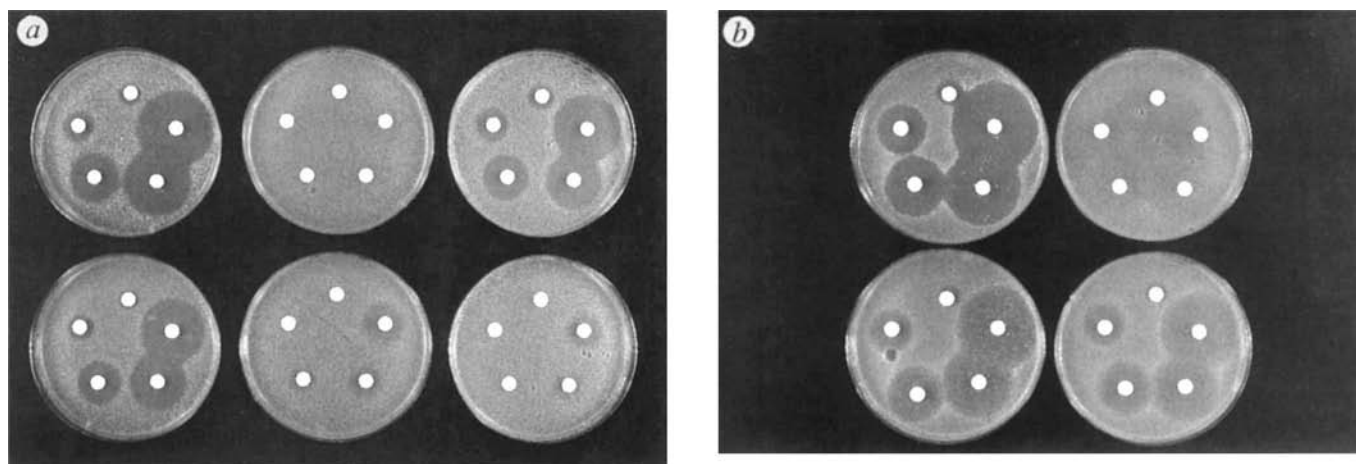


FIG. 3 Phormone response in yeast cells expressing RGS family members. **a**, Phormone response in an *sst2Δ* mutant. An *sst2Δ* mutant (AG57) was transformed with a control plasmid (pBM743) (top left), pVT102U-RGS4 (top middle), pBM743-RGS2 (top right), pBM743-RGS3 (bottom left), pBM743-RGS3T (bottom middle), and pBM743-RGS1 (bottom right). Equal numbers of cells (10^4) were plated in top agar on Sgal-ura plates. Amounts of phormone (α -factor) applied to each disc increase anti-clockwise from the top of the disk (3, 10, 30, 100 and 300 pmol). Plates were incubated at 30 °C for 48 h and then photographed. Relative phormone sensitivity is indicated by the size of the clear zone elicited in response to a given dose of phormone. **b**, Phormone response in cells expressing *SST2*. An *SST2*-expressing strain (AG56) was transformed with a control plasmid (pBM743) (top left), pVT102U-RGS4 (top right), pBM743-RGS3T (bottom left), and pBM743-RGS1 (bottom right). Halo assays were performed as above, except that the amounts of phormone applied to discs (increasing anti-clockwise from the top) were 10, 30, 100, 300 and 1000 pmol. **c**, Effect of RGS4 expression on phormone-induced gene expression. An *sst2Δ* mutant (AG57) contained the *FUS1-lacZ* reporter plasmid pSL307 and either a control plasmid (pVT102U) (diamonds) or pVT102U-RGS4 (squares). Cells were treated for 2 h at 30 °C with the indicated concentrations of α -factor, permeabilized, and assayed for expression of β -gal activity. Each curve is the average of assays performed in duplicate from three independent transformants.

METHODS. Assays of phormone-induced growth arrest and gene expression have been described previously²⁶. Yeast strains used were BC180

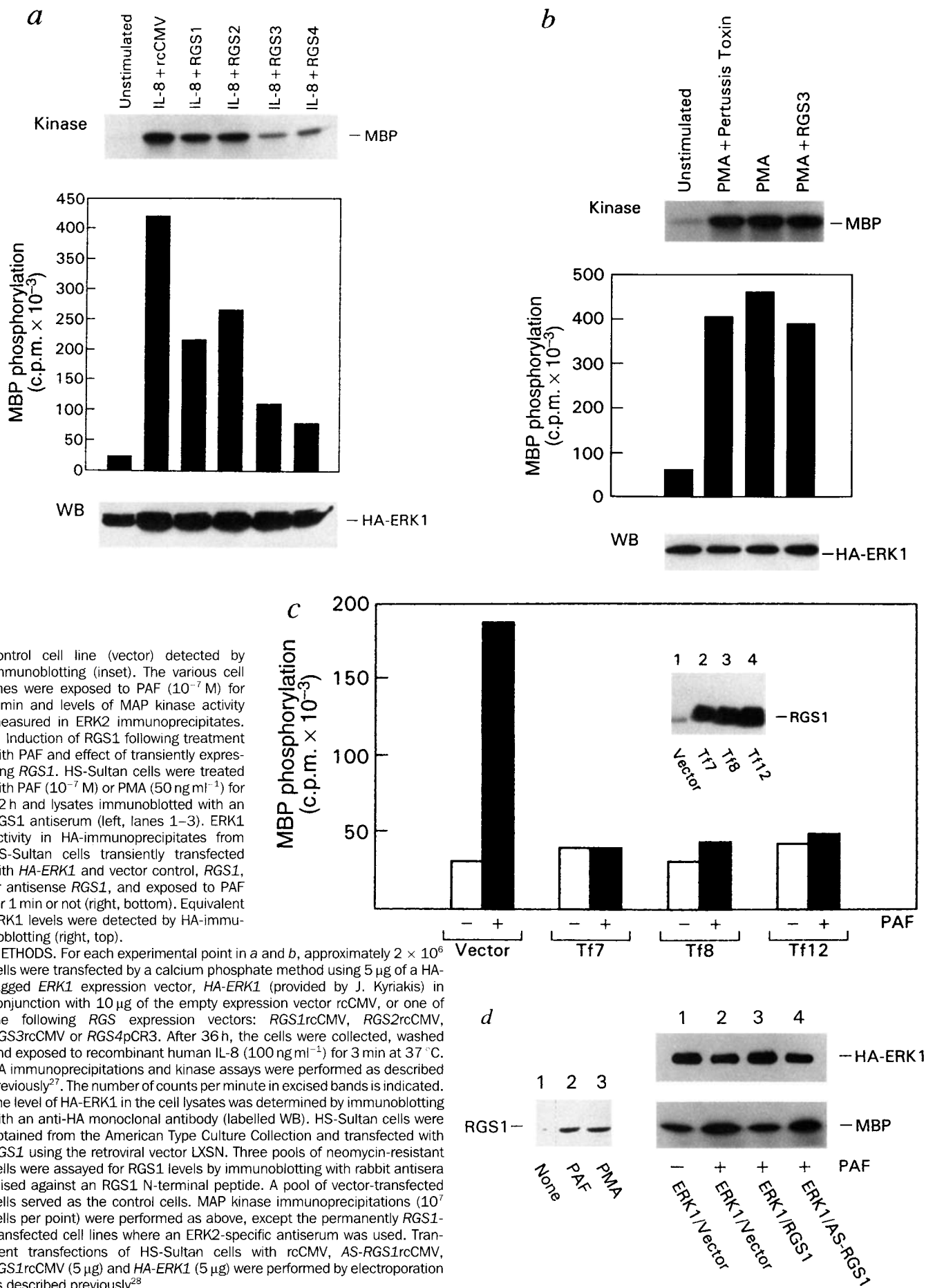
nor *SST2* overexpression attenuated the signal caused by overexpression of a constitutively active *STE11* (*STE11AN*), suggesting that they function upstream of the MAP kinase cassette (data not shown). To initiate signalling more proximally in the pathway, we used a strain lacking G-protein α -subunits that contains a temperature-sensitive *STE5* mutation^{4,9}. In these cells, free G-protein $\beta\gamma$ subunits transmit a constitutive signal that is reversibly blocked downstream by the temperature-sensitive mutation. After releasing the signal blockade, we observed no effect of either *RGS4* or *SST2* overexpression on signal transduction through the pathway. In the control cells, we detected 596 ± 50 Miller units of β -gal activity with the phormone-inducible *lacZ* reporter plasmid pJD11, 6 h after shifting them to the permissive temperature. In those cells in which we overexpressed *SST2* or *RGS4*, we detected 662 ± 79 units and 608 ± 87 units of β -gal activity, respectively, after 6 h. These results demonstrate that *RGS4*, like *SST2* (refs 8–10), requires the G-protein α -subunit to function.

To verify that RGS family members inhibit MAP kinase activation in analogous pathways in mammalian cells, we first examined the activation of extracellular-signal-regulated kinase (ERK) 1 in response to interleukin (IL)-8. We used 293T cells permanently transfected with the IL-8 receptor, a seven-transmembrane helix-domain-containing receptor linked to a trimeric G protein^{18,19}. IL-8 induced a 16-fold increase in ERK1 kinase activity, and the presence of *RGS1*, *RGS2*, *RGS3* and *RGS4* reduced the activity by a factor of 2, 1.6, 4, and 5.3, respectively (Fig. 4a). Increasing amounts of *RGS4* progressively inhibited IL-8-induced ERK1

kinase activity (data not shown). In contrast, *RGS3* failed to impair ERK1 activation by phorbol esters (Fig. 4b) or by an oncogenic, active Raf-1 (data not shown).

We next examined the role of an individual RGS family member in a native mammalian system. We took advantage of the known expression of RGS1 in activated B lymphocytes^{12,13} and determined its effect on signalling through the platelet-activating factor (PAF) receptor, which is expressed on many B cell lines. PAF treatment activates MAP kinase and increases intracellular calcium levels by means of a heterotrimeric G protein(s)^{20,21}. The

FIG. 4 Effect of RGS family members on MAP kinase activation in mammalian cells. **a**, RGS family members impair MAP kinase activation. 293T cells permanently transfected with the IL-8 receptor (6E cells) were transfected with a haemagglutinin (HA)-tagged ERK1 construct in conjunction with empty vector or various RGS expression plasmids. Transfected cells were exposed to IL-8 for 3 min before immunoprecipitation of HA-ERK1 and assay of kinase activity. **b**, RGS3 fails to impair PMA-induced MAP kinase activation. 293T cells were transfected with the HA-tagged ERK1 construct in conjunction with empty expression vector or RGS3 expression vector. The cells were unstimulated (lane 1) or stimulated with PMA (50 ng ml⁻¹) for 20 min (lanes 2–4) before immunoprecipitation of HA-ERK1. The cells in lane 2 were exposed to pertussis toxin for the last 12 h of the culture period. **c**, RGS1 expression inhibits PAF-induced increases in MAP kinase activity. Levels of RGS1 in RGS1-transfected HS-Sultan cell lines (3 separate cell lines labelled Tf7, Tf8 and Tf12) or a



constitutive expression of RGS1 in HS-Sultan cells, a human B-cell lymphoma cell line that lacks significant levels of RGS1, markedly decreased PAF-induced MAP kinase activity (Fig. 4c). In addition, PAF induced RGS1 expression in HS-Sultan cells, and transient expression of RGS1 inhibited PAF-induced increases in MAP kinase activity (Fig. 4d). These results suggest that RGS1 participates in a negative feedback loop to decrease signal transduction through the PAF receptor.

These (and previous) results are consistent with models in which Sst2p and RGS4 (and presumably other RGSs) negatively regulate signalling upstream or at the level of the heterotrimeric G protein^{6,10}. Furthermore, genetic studies in *C. elegans* indicate that an RGS family member, *egl-10*, negatively regulates signalling through GOA-1 (a homologue of the mammalian G_o proteins) at the level upstream of the G protein²². Although, under certain circumstances, *SST2* is required for the rapid degradation of the G-protein α -subunit²³, it is unlikely that regulating the stability of the α -subunit is the normal function of Sst2p or the RGS family members. The Sst2p-dependent degradation of the α -subunit occurred after overexpression of components of the ubiquitin-mediated protein degradation machinery²³. However, in wild-type yeast, steady-state levels of the α -subunit are unaffected by *SST2* (ref. 10) and, similarly, in HS-Sultan cells the levels of α -subunits are unchanged by *RGS1* overexpression (data not shown). In addition, yeast that lack components of the ubiquitin-mediated protein degradation machinery are not supersensitive to pheromone²⁴, and *SST2* overexpression in these mutants promotes rapid recovery from pheromone-induced cell-cycle arrest (data not shown). Although further genetic and biochemical studies are needed to determine how *SST2* and RGSs inhibit G-protein-mediated MAP kinase activation, they probably regulate the function of G proteins directly, because Sst2p and Gpalp associate *in vivo* and *in vitro* (H. Dohlman and J. Thorner, personal communication), and because a mammalian RGS family member has been shown to bind $G\alpha_{13}$ (ref. 25).

It is likely that individual RGS family members preferentially regulate specific G proteins or G-protein-linked signalling pathways. The four characterized family members differed in their patterns of tissue expression and in their ability to impair IL-8 receptor signalling, providing some evidence of selectivity. Further specificity may be achieved by regulating their induction. As with *SST2*, RGS family members may be induced by signals generated through G-protein-linked receptors to trigger a desensitization mechanism. □

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RNA binding and translational suppression by bicoid

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THE anterior determinant bicoid (*bcd*) of *Drosophila* is a homeo-domain protein. It forms an anterior-to-posterior gradient in the embryo and activates, in a concentration-dependent manner, several zygotic segmentation genes during blastoderm formation¹⁻⁴. Its posterior counterpart, the homeodomain transcription factor caudal (*cad*)⁵⁻⁷, forms a concentration gradient in the opposite direction, emanating from evenly distributed messenger RNA in the egg. In embryos lacking *bcd* activity as a result of mutation, the *cad* gradient fails to form and *cad* becomes evenly distributed throughout the embryo⁸. This suggests that *bcd* may act in the region-specific control of *cad* mRNA translation. Here we report that *bcd* binds through its homeodomain to *cad* mRNA *in vitro*, and exerts translational control through a *bcd*-binding region of *cad* mRNA.

Maternal *cad* mRNA is evenly distributed in the early cleavage-stage embryo (Fig. 1a), and *cad* forms a posterior-to-anterior concentration gradient before the initiation of zygotic gene expression^{6,7} (Fig. 1b, d). In the absence of functional *bcd* expression (Fig. 1c, d), *cad* remains evenly distributed in the embryos⁸ (Fig. 1e-g). To see whether *cad* mRNA may represent a direct target of *bcd*, we performed crosslinking and filter-binding experiments involving nuclear extracts from *Drosophila* embryos and bacterially expressed *bcd*.

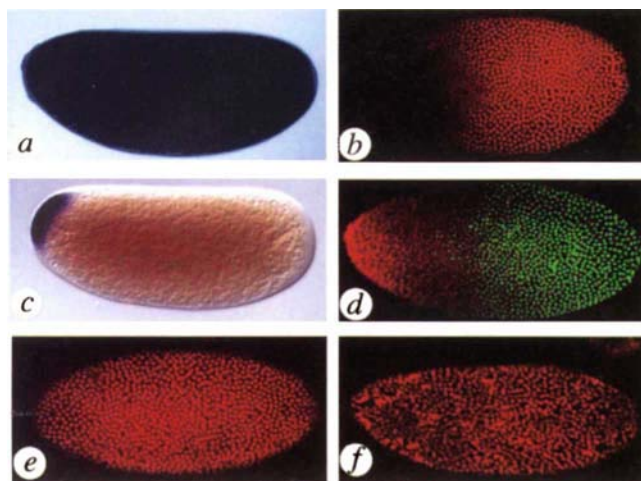
Crosslinking experiments indicated specific binding of labelled *cad* mRNA (Fig. 2a) to three different proteins, p125 and the protein doublet p83/p71, present in nuclear extracts from wild-type embryos (Fig. 2b). The p83/p71 doublet could be immunoprecipitated with anti-*bcd* antibodies (Fig. 2b): *cad* mRNA binding was also seen in crosslinks with recombinant *bcd* (not shown). However, the p83/p71 doublet was absent from nuclear extracts of embryos obtained from *bcd* mutant females (Fig. 2b) and from *bcd*-depleted nuclear extracts of wild-type embryos (data not shown). The p125 protein was found in both cases binding to the region 1,916–2,028, which overlaps the maternal and zygotic polyadenylation signals (Fig. 2d). Thus the p83/p71 doublet, which binds to *cad* mRNA *in vitro*, is *bcd*.

The binding of *bcd* occurs within a 120-nucleotide region of *cad* mRNA (position 1,350–1,470; see Fig. 2a; position 1 is the first nucleotide of the start codon⁹), which extends into the 3' untranslated region (Fig. 2c). The *bcd* protein did not bind to other regions of *cad* mRNA, nor did it bind to *cad* mRNA of the lower dipteran species *Clogmia albipunctata* (U.S.-O., unpublished results) which is thought to lack *bcd* as an anterior morphogen. We refer to the *bcd*-binding sequence interval 1,350–1,470 of *cad* mRNA in *Drosophila* as the *bcd* binding region (BBR).

To identify the RNA-binding domain of *bcd*, we performed filter-binding assays using truncated forms of *bcd*. The results summarized in Fig. 2e indicate that truncated *bcd* containing the

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g

Allele	Molecular lesion	Caudal gradient
Wild type	—	gradient formed; see b, d
<i>bcd^{E1}</i>	amino acid interval 151 to 207 deleted; followed by frame shift	no gradient formed; see e
<i>bcd^{E2}</i>	homeodomain deleted	no gradient formed
<i>bcd^{GB}</i>	stop codon in position 153	no gradient formed
<i>bcd⁰⁸⁵</i>	stop codon in position 179	no gradient formed
<i>bcd^{E5}</i>	stop codon in position 259	no gradient formed, anterior retraction

FIG. 1 *bcd*-dependent formation of the *cad* gradient. *a*, Maternal *cad* mRNA distribution, and *b*, corresponding *cad* gradient in wild-type embryos. Note the formation of an posterior-to-anterior *cad* gradient, while the mRNA is evenly distributed. *c*, *bcd* mRNA localized to the anterior pole, and *d*, the resulting *bcd* gradient (red) overlapping the *cad* gradient (green). *e*, *cad* distribution in embryos derived from homozygous *bcd^{E1}* females; no gradient is formed. *f*, *cad* distribution in embryos derived from *bcd^{E1}* females bearing a *bcd* transgene which lacks the homeodomain. The modified *bcd* lacking the homeodomain forms a stable protein gradient (data not shown). Note that, although this embryo is slightly older than the others, no *cad* gradient is formed. This suggests that the *bcd* homeodomain is necessary for *cad* gradient formation. Orientation of embryos is anterior to the left, dorsal up; embryos were at the early syncytial blastoderm stage. *g*, Summary of different *bcd* mutants that fail to form the *cad* gradient. Note that *bcd* is forming a normal or reduced gradient in *bcd⁰⁸⁵* and *bcd^{E5}*, but no protein was found in the other mutants³. Molecular lesions in *bcd^{21,22}* are indicated.

METHODS. RNA whole-mount *in situ* hybridization²³ with *cad* antisense RNA (*a*), DNA whole-mount *in situ* hybridization²⁴ with *bcd* DNA probes (*c*), anti-*cad* antibody stainings⁸ and anti-*bcd* monoclonal antibody³ (BCD MAB23, ATCC, Rockville, MD) were done as described in the references. Embryos stained with fluorescent secondary antibodies (fluorescein and Cy3, respectively) were examined by laser-scanning microscopy and the images processed by NIH1.54 software.

FIG. 2 *In vitro* binding of *bcd* to *cad* mRNA. *a*, *cad* mRNA showing open reading frame (ORF, filled bar), 3' UTR, maternal (*mat*) and zygotic (*zyg*) polyadenylation sites⁹ and protein-binding regions (hatched boxes; see *b-d*); numbers start with first ATG in *cad* cDNA⁹. P, *Pst*I; S, *Ssp*I; R, *Eco*RI. *b*, *In vitro*-crosslinked ³²P-labelled full-size *cad* mRNA to nuclear proteins (NP) from *Drosophila* embryos; no prot, control; wild type, wild-type NP; *bcd^{E1}*, NP from embryos of homozygous *bcd^{E1}* females. Arrows indicate crosslinked proteins (lane 'wild type'), bar marks unspecific binding to yolk protein (varies from experiment to experiment); *M_r*(K) refers to markers. p83/p71 is absent in NP of *bcd^{E1}*, while p125 is crosslinked (lane '*bcd^{E1}*'). p83/p71 immunoprecipitate with anti-*bcd* antibodies (lane 'antibody precipitate'). *c*, *d*, *cad* region 1,350–1,470 and 1,916–2,028 (hatched boxes in *a*) bind to p83/p71 (*c*) and p125 (*d*), respectively (0, 5: µg of wild-type NP). Other regions of *cad* mRNA did not bind (not shown). Thus *bcd* binds to region 1,350–1,470 (BBR; see text). *d*, Filter-binding assay with ³²P-labelled full-size *cad* mRNA. Full-size *bcd* (*bcd¹⁻⁴⁸⁹*; numbers according to ref. 21) and truncated forms bind only if the homeodomain (filled bar) is present. Note that the *bcd* C terminus (homologous to RNA-binding proteins²⁵), caudal, orthodenticle, antennapedia homeodomain²⁶ and bovine serum albumine do not bind.

METHODS. UV crosslinking was performed using a total energy of 4,000 µJ (ref. 27). Nuclear extracts were from 0–3-hour-old wild type or embryos²⁸. Binding reactions (5–15 µg of protein; 2.5–5 × 10⁵ d.p.m. of indicated ³²P-labelled RNA; 10 min, 20 °C) were done in 20 µl binding buffer (10 mM HEPES-K⁺ buffer, pH 7.6, 25 mM KCl, 1.25 mM MgCl₂, 2 mM DTT, 1 mM ATP, 5 mg ml⁻¹ heparin, 5% glycerol and 10 µg ml⁻¹ poly dIdC, 100 µg l⁻¹ yeast total RNA and 1 mg ml⁻¹ *E. coli* tRNA as unspecific competitor). Immunoprecipitation was performed with monoclonal anti-*bcd* antibodies³ (ATCC, Rockville, MD) (1 h, 4 °C) and agarose-coupled anti-mouse IgG antibodies (several hours; 4 °C). Crosslinks were separated by SDS-PAGE. For the filter-binding experiments, 100 pmol of recombinant *bcd* (full length or truncations), antennapedia homeobox, caudal²⁶ or orthodenticle were bound in native conditions to nitrocellulose membranes, blocked in binding buffer (1 h, 4 °C; see above). Filters were incubated (30 min, 4 °C) in binding buffer containing 1 × 10⁶ d.p.m. ml⁻¹ to 2 × 10⁶ d.p.m. ml⁻¹ ³²P-labelled full-size *cad* mRNA, washed three times in excess binding buffer and analysed by phosphor imager.

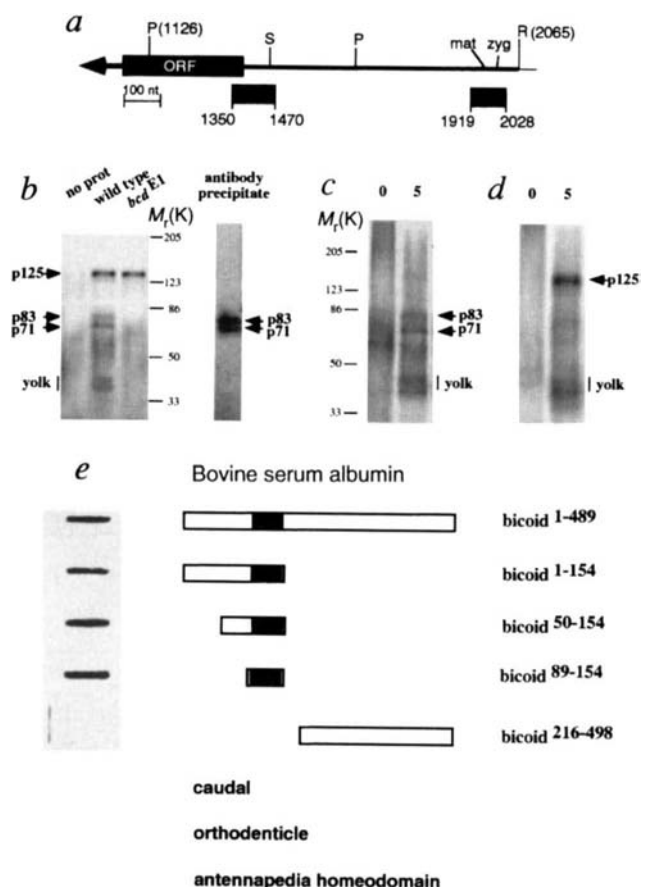
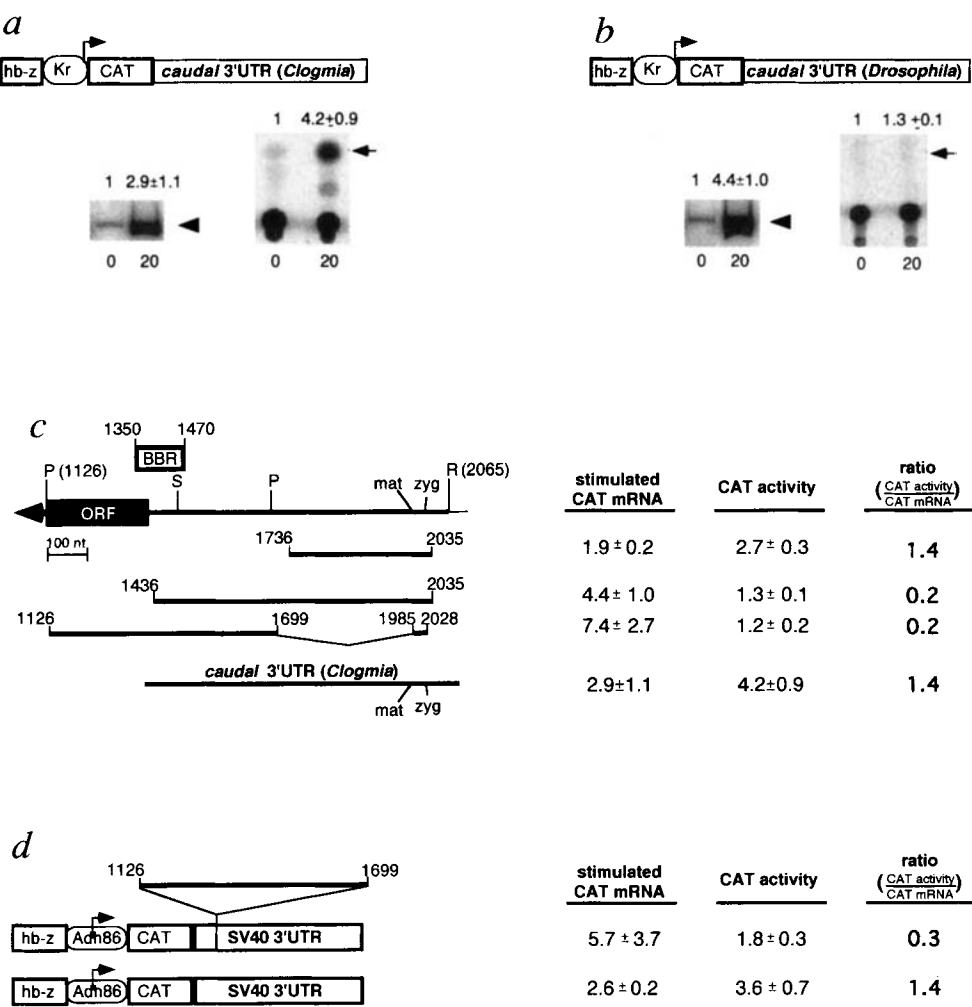
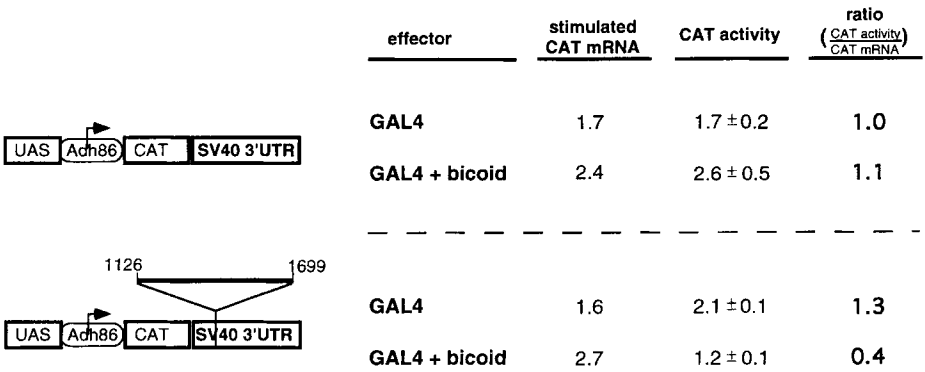


FIG. 3 The BBR reduces translation efficiency in transfected *Drosophila* Schneider cells¹¹. *a*, CAT reporter gene containing the 3' UTR of *Clogmia* *cad* mRNA and the *bcd*-dependent *hunchback* enhancer¹² (*hb-z*) in front of the *Krüppel* promoter²⁹ (top). CAT mRNA production (left, arrowhead) in the absence ('0') or presence of *bcd* (20 µg pActBcd effector plasmid¹²; '20') and corresponding CAT activities (right, arrow). *b*, CAT reporter gene containing the 3' UTR of *Drosophila* *cad* mRNA (region 1,436–2,035; see *c*) (top); the 3' UTRs differ except for the p125-binding region (see Fig. 2; U.S.-O., unpublished data). CAT mRNA production (arrowhead) in the absence ('0') or presence of *bcd* ('20') (left) and corresponding CAT activities (arrow, right). *c*, Diagram of *Drosophila* *cad* mRNA (left, details in Fig. 2) and portions of it serving as 3' UTRs (for numbering see Fig. 2). Stimulated CAT mRNA and corresponding CAT activity (right, examples shown in *a* and *b*). The presence of part of *bcd*-binding region (BBR, see Fig. 2 and text) and region 1,126–1,699 cause reduced CAT activity (Fig. 2*c*). Thus the overlap between the two (region 1,436–1,699) suppresses translation (see also Fig. 4). More 3' portions of the 3'UTR also cause weak suppression (not shown); region 1,736–2,035 does not. *d*, CAT reporter genes containing the SV40 3' UTR with (top row, left) or without (below) region 1,126–1,699 (see *c*) and the *Drosophila* *Adh86* (ref. 11) promoter; *bcd*-stimulated CAT mRNA and corresponding CAT activity in response to 1 µg of *bcd* effector plasmid (right). METHODS. Transfections of the *Drosophila* Schneider cells and CAT assays were as described¹¹. Quantitative RT-PCR¹³ was done with primers 5'-CCACTGGGATTATACACCGTTGGATATATCC-3' and 5'-TTGCGAATATATGTGT-



AGAACTTGCCGG-3'. Results are expressed as stimulated CAT mRNA and CAT activity over basal activities. Mean values and standard deviation derived from at least four experimental series. Region 1,126–1,699 of *cad* mRNA (PstI fragment) was inserted into the PstI site of the SV40 3' UTR.

FIG. 4 *bcd*-dependent translational suppression of BBR-containing mRNA in transfected *Drosophila* Schneider cells. *a*, Left, diagram of the CAT reporter gene constructs containing the 3' UTR of SV40 with (top) or without (bottom) the BBR of *cad* mRNA. Reporter genes were as shown in Fig. 2*d*, except that GAL4-dependent upstream activating sequences (UAS)¹⁴ were used instead of the zygotic *hunchback* promoter. Right, effector-dependent stimulation of CAT mRNA and corresponding CAT activity in *Drosophila* Schneider cells¹¹ which were co-transfected with GAL4-producing effector plasmid¹⁴ ('GAL4') or with GAL4- and *bcd*-expressing effector plasmids ('GAL4 + *bicoid*'). METHODS. Cells were co-transfected with 1 µg reporter gene plasmid and 10 µg GAL4-producing effector plasmid¹⁴, or with a combination of 10 µg GAL4-producing effector plasmid¹⁴ and 5 µg *bcd*-expressing effector plas-



mid¹² as described previously¹¹. Each value represents the mean of at least four independent transfection experiments. Other methods are described in the Fig. 3 legend.

homeodomain is sufficient for the binding to *cad* mRNA; other regions of the protein, or other homeodomain proteins such as *cad*, orthodenticle and the homeodomain of antennapedia, did not bind. That the *bcd* homeodomain interacts with *cad* mRNA *in vitro* is consistent with the *in vivo* observation, suggesting that the homeodomain of *bcd* is necessary for the formation of the *cad* gradient (Fig. 1f,g). The failure of other homeodomain proteins to bind *cad* mRNA does not rule out the possibility that they possess RNA-binding properties as preceded by zinc-finger-type transcription factors¹⁰.

To assess the functional significance of the *in vitro* interaction between *bcd* and *cad* mRNA, we performed co-transfection experiments with *Drosophila* Schneider cells¹¹. We used chloramphenicol acetyl transferase (CAT) reporter gene constructs¹¹ containing different 3' UTR sequences (Fig. 3). The reporter gene constructs were set under the control of the *bcd*-responsive, *cis*-acting element of the zygotic *hunchback* promoter¹². After co-transfection with *bcd*, we determined the stimulated transcription of CAT mRNA by reverse transcription-polymerase chain reaction (RT-PCR)¹³, and the translation of the CAT mRNAs with the different 3' UTRs by assaying CAT activity¹¹.

CAT mRNAs containing the *Drosophila cad* 3' UTR sequences or the regions including the BBR produced about sevenfold less CAT activity than CAT mRNA containing the *cad* 3' UTR sequences of *Clogmia* or the 3' half of the 3' UTR of *Drosophila cad* (Fig. 3a-c). CAT mRNA containing the BBR within the simian virus 40 (SV40) 3' UTR¹¹ were also translated less efficiently than those containing only the SV40 3' UTR. These results suggest that *bcd* may not only act as a transcriptional activator in the assay system, but also reduce the translation efficiency of the BBR-containing mRNAs.

To show that *bcd* does indeed suppress the translation of BBR-containing mRNA, we examined reporter gene constructs containing the SV40 3' UTR with or without the BBR that were transcriptionally activated by the yeast GAL4 transcriptional activator¹⁴. In the absence of *bcd*, the GAL4-stimulated CAT mRNAs were translated with similar efficiency (Fig. 4). In the presence of *bcd*, however, the translation of the BBR-containing CAT mRNA was significantly reduced, but the translation of CAT mRNA without the BBR was not significantly affected. This shows that *bcd* suppresses the translation of BBR-containing mRNA.

Our results are consistent with the argument that the anterior determinant *bcd* acts not only as a transcriptional activator but also functions in a manner analogous to the key components of the posterior maternal pattern-organizer system¹⁵⁻²⁰, which acts by translational repression through the activities of *nanos* and *pumilio*¹⁵⁻²⁰. After binding the *pumilio* protein and a 55K protein to the *nanos*-response element within the 3' UTR of *hunchback* mRNA^{17,20}, the *nanos* protein is thought to interact with this 'landing pad' to provide region-specific translational repression of the evenly distributed maternal *hunchback* mRNA¹⁵⁻¹⁹. This allows for the activation of the posterior gap genes *knirps* and *giant* in response to *cad*⁵. Thus, while in the posterior region of the embryo translational and transcriptional control are conducted through separate components, the anterior determinant *bcd* can exert both regulatory functions. Our findings also imply that the asymmetric distribution of the key components of the anterior and posterior maternal pattern-forming systems, which was thought to be set up independently^{1,2}, is linked through the dual function of *bcd*. □

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ADDENDUM

Identification of the breast cancer susceptibility gene *BRCA2*

Richard Wooster, Graham Bignell, Jonathan Lancaster, Sally Swift, Sheila Seal, Jonathan Mangion, Nadine Collins, Simon Gregory, Curtis Gumbs, Gos Michlem, Rita Barfoot, Rifat Hamoudi, Sandeep Patel, Catherine Rice, Patrick Biggs, Yasmin Hashim, Amanda Smith, Frances Connor, Adelgeir Arason, Julius Gudmundsson, David Ficenc, David Kelsell, Deborah Ford, Patricia Tonin, D. Timothy Bishop, Nigel K. Spurr, Bruce A. J. Ponder, Rosalind Eeles, Julian Peto, Peter Devilee, Cees Cornelisse, Henry Lynch, Steven Narod, Gilbert Lenoir, Valdgardur Egilsson, Rosa Bjork Barkadottir, Douglas F. Easton, David R. Bentley, P. Andrew Futreal, Alan Ashworth & Michael R. Stratton

Nature **378**, 789-792 (1995)

THE sequence for the *BRCA2* gene is now available at the web page of the Institute of Cancer Research, address <http://www.icr.ac.uk/molcarc/brca2.htm>, as well as from Genbank. □

ERRATUM

Mechanosensory signalling in *C. elegans* mediated by the GLR-1 glutamate receptor

Andres V. Maricq, Erin Peckol, Monica Driscoll & Cornelia I. Bargmann

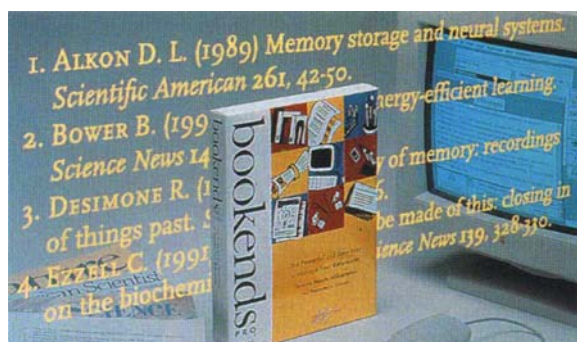
Nature **378**, 78-81 (1995)

FIGURES 3 and 4 of this Letter were inadvertently transposed during the production process; the legend to Fig. 3 therefore describes the figure published as Fig. 4 and vice versa. □

Digital dialogue

This is where cyber-buffs can do their digging for information, such as on-line access to chemical viewing software and biological review journals, as well as statistical and graphing software.

WESTING SOFTWARE has announced Bookends Pro version 3.2 for Macintosh computers, a complete **bibliography management system**. It has been designed for scientists, historians, scholars in the humanities and graduate students who need to track and cite reference materials for research and publication. The program is said to be accelerated for the Power Mac and features a redesigned interface, expanded print capabilities and automatic updating. Importing downloaded reference



Bookends Pro bibliographic management program.

files and scanning a document for citations is said to be much faster with the improved find-and-replace feature. Other features include the ability to edit any field references (or just those in the 'hits list'), to handle editor labels in a bibliography, to create templates that allow import reference information from on-line services or CD-ROMs, to communicate with a linked word processor, to create new bibliography formats, and to attach *ashy* files (word processor document, spreadsheet, PDF file) and pictures (PICT and bitmapped graphics).

Reader Enquiry No. 100

Molecular imaging

Synopsys Scientific Systems, a provider of chemical information software and database solutions, has announced the availability of its Accord **chemistry viewer**, free on the Internet (www.synopsys.co.uk) for personal, academic and registered corporate use. The viewer can be installed as a helper application within web browsers, such as Netscape or Mosaic, to retrieve and depict chemical structures and reactions from web servers that are stored in a host of chemical formats, including SMILES strings, ChemDraw files, MDL Molfiles and Rxnfiles. It also makes it pos-

sible to visualize structures stored as MIME attachments in Internet mail messages. In addition, publication-quality chemical diagrams produced by the viewer can be transferred via the clipboard to other desktop applications quickly and easily for inclusion in internal reports.

Reader Enquiry No. 101

Biosym/Molecular Simulations has introduced Insight II version 95.0, designed specifically for **molecular visualization**. It has improved visualization capabilities with solid molecular surfaces, ray-casting, Richardson diagrams, high-resolution hardcopy and image mapping. New products released with this version include Ludi/ACD, Affinity and Xsight. Ludi/ACD is a subset from MDL's 50,000-compound chemical database, which expands the range of compounds and molecular diversity for exploring proposed ligand fragments.

Affinity is a new module for rapid automated docking of small molecules into protein active-sites. Completing the trio, Xsight is a new protein crystallography module that resulted from the successful completion of the macromolecular crystallography focus group. Other modules with improvements include Sketcher, Felix, NMRchitect, search/compare and homology.

Reader Enquiry No. 102

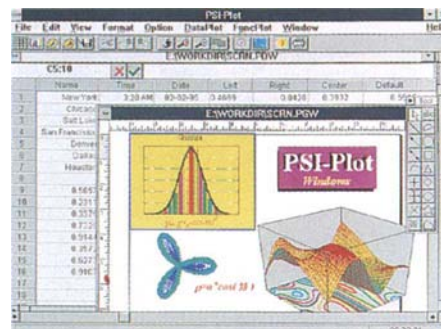
Statistical survey

Now available from Statsoft is Statistica 5.0, which automatically configures for either Windows 3.1 or Windows95. The program is said to offer a wide selection of basic and advanced **statistical and graphical procedures for science**, business and engineering applications. The new release contains a number of enhancements and new procedures, for example, the structural-equation modelling and path-analysis module, and the expanded experimental design, process-analysis and quality-control modules. The optimized user interface offers programming tools, such as Statistica Basic, which is said to be an integrated, easy-to-use programming language that can be used for a variety of applications

ranging from simple data transformations to the building of complex, permanent extensions to the computational, graphics and data management procedures. Routines written in other programming languages can also be integrated.

Reader Enquiry No.103

The Windows version of PSI-Plot from Poly Software has enhancements that are designed to make it easier and more convenient to **analyse technical data, create and edit graphs**. The program provides a comprehensive data sheet that performs complete statistical analysis (*t*-test, *F*-test, ANOVA, regression), data transformation and interpolation, digital signal processing (filter, window, smooth), linear and nonlinear curve fitting, model development and ordinary differential equation solver. Data can be exchanged to and from popular file formats, including Lotus 1-2-3, dBase, QuattroPro, DIF, Microsoft Excel and ASCII. Its graphing



PSI-Plot for analysing and creating graphs.

utilities include drawing tools and a simple equation editor. It also offers many two-dimensional and three-dimensional plot types, including scatter/line graphs in both cartesian and polar coordinates, vector plot, surface (mesh) graphs, bar charts, box, high-low, step, Smith chart and ternary plot.

Reader Enquiry No. 104

On ROM

Current Biology now publishes its **journals on CD-ROM**, ensuring the accessibility of hundreds of reviews, figures, tables and interactive 'kinemages', as well as thousands of relevant references. Biology Current Opinions with evaluated Medline contains a comprehensive range of biol-

ogy review journals from cell biology to neurobiology, biotechnology to structural biology, and immunology to genetics and development. **Macromolecular Structures: Database and Reviews** combines Macromolecular Structures, a directory of all new reported macromolecular structures, and **Current Opinion in Structural Biology** is a review of all major developments in the field with full Medline references and a bibliographic database. These are cross-referenced to form a flexible resource. <http://BioMedNet.com/>
Reader Enquiry No. 105

The first release of Linux for Astronomy, a **CD-ROM containing a collection of astronomy software**, compiled for use with the Linux operating system, is now available from Random Factory. The CD-ROM includes the popular astronomical data-processing packages. General purpose packages, such as AIPS, IRAF, MIDAS, including CCD image processing, spectral analysis, photometry, astrometry and more. A comprehensive set of on-line documentation is provided, along with a searchable index. A searchable database of 6,500 astronomy-related World Wide Web resources is also included.
Reader Enquiry No. 106

Data acquisition

GraphPad Software has released a **scientific graphing and data analysis program**, Prism version 2.0 for Windows. This version adds biostatistical data analyses, such as *t*-tests, one-way ANOVA, nonparametric tests and analyses of contingency tables, in an attempt to make Prism a complete biostatistics, curve-fitting and scientific graphing package. Users can summarize an experiment by combining multiple graphs and portions of data tables and analysis results on a single page. There is said to be no need to switch back and forth between multiple programs. Scientists can analyse their data with Prism's working demo, available on the WWW at <http://www.graphpad.com/>
Reader Enquiry No. 107

Bio Image has released 2-D Analyzer 6.1 software for Sun SPARCstations for the analysis of spots and gels. The software is said to **find, quantify and match spots quickly on any number of scanned gel images** within a database. Querying features can find the spots of interest, study and report the data in any of a variety of formats, including charts, histograms and spot maps. Bio Image also offers a multiplatform version of analyser in its 'intelligent quantifier' (IQ) software for PC and Macintosh. The IQ software allows users automatically to detect, quantify and report spot data from scanned images of two-dimensional gels.
Reader Enquiry No. 108

VoxBlast is now offering a native version for Power Mac, as well as new Windows95 and NT programs. This VayTek **software for rendering, measuring and presenting three-dimensional data** is said to run approximately three times faster in the new native version for Power Macs. The manufacturer claims that with the increase in speed, the Power Mac can match many UNIX workstations in accepting a stack of registered two-dimensional images for creation of three-dimensional projections. It offers arbitrary slicing planes, true three-dimensional measurements, surface extraction, polygon rendering, flood-fill capabilities and volumetric calculations. Sophisticated lighting models, movie generation features and palette editors are included in the program. Presentation and evaluation of data is enhanced when the user can incorporate colour, transparency, motion and lighting — pseudocolouring is available from a palette of 16 million colours. All of these features can be used in self-scripted movie loops. The program supports TIFF files, true 24-bit RGB rendering, 16-bit dual-mode rendering and volume densitometry.
Reader Enquiry No.109



VoxBlast software.

Graphics gallery

National Instruments has released the new LabView demonstration packages for Windows and Windows NT, Macintosh/Power Macintosh, Sun SPARCstations, and Hewlett-Packard 9000-series 700 workstations. Users of the demonstration can automatically run a variety of applications involving **instrument control and data acquisition, analysis and presentation** for test and measurement and process monitoring and control applications. Information about the program, customer education, technical support and add-on tool kits is also available at the click of a button. The new demonstration package, based on version 3.1.1 of the software package, features a tutorial-style manual, as well as a menu-based system so that users can view a variety of applications and information.
Reader Enquiry No. 110

Adept Scientific has announced the latest version of its data acquisition software, Workbench for Windows. It is said to deliver high-speed data acquisition and to allow users to set up complex **data acquisition, analysis and control systems on-screen**. The manufacturer states that programming skills are not needed to use this program. Software versions of signal-conditioning units, meters, chart recorders and most hardware items associated with data acquisition are all included as stan-

dard. The program provides a comprehensive library of colour graphic displays, including analog meters, charts and waterfall frequency plots, all of which can be connected to data inputs with simple mouse-driven operations. The software provides DDE links to make exporting data to other applications a simple task. The manufacturer states that the system can support real-time display at rates of up to 5 kHz. It now enables data from high-speed boards to be streamed direct to disk at 100 kHz. Additionally, the software's 'burst' acquisition mode now provides transient capture at rates of up to 1 MHz.
Reader Enquiry No. 111

SciTech has announced the publication of **Software for Science volume 30**, a comprehensive **catalogue of scientific, engineering and technical software** for DOS, Windows, Macintosh and UNIX workstations. This edition includes 100 colour pages with over 100 new Windows95 products, in addition to the 1,850 other products already listed in the catalogue. It addresses the recent Windows95 announcement with articles for scientific, engineering and technical professionals.

Reader Enquiry No. 112 □

These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.

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